NODULATION, GROWTH AND WATER USE OF CHICKPEAS (*Cicer arietinum L.*)

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(*Cicer arietinum L.*)

ABSTRACT

The potential of chickpea as an alternative rotation crop in Canterbury has already been established. Experiments were therefore planned to investigate the effects of inoculation, additional nitrogen fertilizer and supplemental watering on its nodulation and growth and the suitability of sowing in either winter or spring.

Three experiments were carried out using *kabuli* chickpeas; two on a Templeton silt loam soil in the field and one in the glasshouse. The first field trial was sown on two dates (3 July and 30 September), provided with four rates of nitrogen fertilizer (0, 15, 45 and 90 kg N/ha) and three rates of *Rhizobium* inoculant (zero, recommended rate and twice the recommended rate). Experiment two was carried out in the glasshouse with three levels of supplemental watering (1/3 field capacity, 2/3 field capacity and field capacity), two rates of nitrogen fertilizer (0 and 90 kg N/ha) and either inoculated with the recommended rate of *Rhizobium* inoculant or not inoculated. In the last field trial, the chickpea was inoculated and sown on three dates (9 July, 9 August and 14 September) with (90 kg N/ha) or without additional nitrogen fertilizer.

Under both field and glasshouse conditions, nodulation response to *Rhizobium* inoculation was poor with the number of nodules initiated per plant rarely exceeding 10. Added fertilizer nitrogen reduced the number of nodules per plant by 50% in the early part of the 1992/93 season. In the glasshouse, the reduction in nodule number in the presence of additional nitrogen persisted throughout plant growth such that at 30, 45 and 60 DAS, nodule number per plant was reduced by 84, 59 and 53% respectively. Seed reserve mobilization was enhanced by both additional nitrogen and supplemental watering. The cotyledons of the fully watered plants weighed 50 and 77% less than those of the plants maintained at 1/3 field capacity at 30 and 45 DAS respectively. At 30 DAS the plants receiving additional nitrogen had cotyledons weighing 31% less than those not provided with additional nitrogen.
In the glasshouse added nitrogen reduced root dry weights from 0.16 g/plant to 0.13 g/plant

at 30 DAS and from 0.23 g/plant to 0.19 g/plant at 45 DAS. However, it increased branch number and leaf number per plant by between 21 and 87% and 17 and 48% respectively between 30 and 60 DAS. Green area per plant was increased from 72.3 cm^2 to 93.3 cm^2 at 45 DAS and from 66.8 cm^2 to 110.7 cm^2 at 60 DAS and shoot dry weight from 0.92 g/plant to 1.30 g/plant at 60 DAS in the presence of additional nitrogen. For plants maintained at field capacity the increase in root dry weight was between 33 and 180%, that of leaf number between 18 and 74% and green area between 52 and 226%. Shoot percent nitrogen increased by 30% in the presence of additional nitrogen and by 32% when the plants were inoculated with the recommended rate of *Rhizobium* inoculum.

Seed yield averaged 2.87 t/ha with a harvest index (HI) of 0.29. Harvest index was significantly ($p<0.05$) increased by *Rhizobium* inoculation from 0.26 to 0.31 at zero and double the recommended rate respectively. It was also increased significantly ($p<0.001$) as sowing date was delayed from winter (0.25) to spring (0.33). Maximum dry matter yield declined as the sowing date was delayed from 11.2 t/ha when sown in July to 9.5 t/ha when sown in September. Added fertilizer nitrogen increased leaf area index (LAI) from 2.7 without additional nitrogen to 3.7 when 90 kg N/ha was provided and total intercepted photosynthetically active radiation (PAR) from 725 MJ PAR/m^2 to 760 MJ PAR/m^2 at 0 and 90 kg N/ha respectively. Total PAR intercepted declined as sowing date was delayed with the July sowing intercepting 20% more PAR (810 MJ PAR/m^2) than the September sowing (675 MJ PAR/m^2). Total dry matter accumulation was closely related to PAR intercepted throughout the growing season and on average 1.64 g of dry matter was produced for every MJ of PAR intercepted.

This work suggests that farmers are unlikely to obtain a yield advantage with winter sowing of chickpeas but sowing about late September to early October will give maximum yields. *Rhizobium* inoculation is necessary to ensure nodulation, however more work is necessary to determine suitable methods of inoculation. Water stress will reduce chickpea growth and dry matter yield in the glasshouse, primarily due to a reduction in green area. Chickpea crops do not fix adequate nitrogen for their own use, however in the field there was little indication that added nitrogen benefited crop growth.

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LIST OF SYMBOLS

Symbol		Units
C	final crop weight	g/m^2
C_o	counts above the canopy	-
C_b	counts below the canopy	-
C_m	maximum crop growth rate	$g/m^2/d$
DAS	days after sowing	d
DM	dry matter	t/ha or g/m^2
DUR	duration of period of exponential growth	d
F_p	fraction of radiation intercepted	-
HI	harvest index	-
LAI	leaf area index	-
PAR	photosynthetically active radiation	MJ/m^2
R^2	coefficient of determination	%
R_s	relative sensitivity	-
T	fraction of light transmitted through the canopy	-
τ_p	transmission coefficient for PAR	-

τ_t	transmission coefficient for total solar radiation	-
WMAGR	weighted mean absolute growth rate	$g/m^2/d$
Y	yield	g/m^2 or kg/ha

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CHAPTER 1

INTRODUCTION

Peas (*Pisum sativum* L.) have traditionally been the main grain legume cultivated in New Zealand (Jermyn, 1988), but emphasis is now shifting towards the production of other grain legumes initially considered of minor importance. Chickpea (*Cicer arietinum* L.) falls into this category and research into its production in New Zealand is fairly recent (Hernandez, 1986).

With an average worldwide seed yield of around 700 kg/ha (Nene, 1987), chickpea would remain a marginal crop (Jodha and Subba Rao, 1987). Previous work in Canterbury with *kabuli* chickpeas however indicates that a seed yield from 2.7 to 3.5 t/ha is achievable (Hernandez, 1986; McKenzie *et al.*, 1992). If grown on a commercial basis therefore, this could offset current imports estimated at 69 tonnes and leave a surplus for export to European and Asian markets (Hill, 1991). Considerable work on lentils has shown that early sowing increases seed yield (McKenzie and Hill, 1990; Turay, 1993). Similar results have been obtained in chickpea grown in the Mediterranean region (Saxena and Goldsworthy, 1988). However Hernandez (1986) has shown that spring sown chickpeas may yield higher than autumn sown chickpeas. There is therefore a need to define the appropriate sowing date for chickpea within the Canterbury region.

Global environmental trends are in favour of low input sustainable agricultural systems in which legumes provide the bulk of the nitrogen inputs (White, 1991). If chickpea is to become a useful component of such a system, it must be able to enhance soil nitrogen under the range of commonly encountered soil inorganic nitrogen levels (Jessop *et al.*, 1984). However, much remains to be learned about the practical aspects of chickpea inoculation (Somasegaran *et al.*, 1988). Also data on the amount of nitrogen fixed by chickpeas in the Canterbury environment is lacking. Other studies have indicated an inability of chickpeas to fix sufficient nitrogen for maximum growth (Hernandez and Hill, 1984). Therefore, farmers need to know the level of nitrogen fertilizer that can be added to ensure maximum crop growth without suppressing nodulation and nitrogen fixation.

Canterbury, where seventy five percent of New Zealand's crop production area is situated (Jermyn, 1988), experiences annual droughts in the months of January and February (McKenzie and Hill, 1990). Most traditional crops therefore require relatively large amounts of irrigation without which yield losses can be high. During the 1988/89 droughts, the region's farmers collectively suffered a $NZ 800 million loss (Morgan, 1991). Understanding the water requirements of newly introduced crops like chickpea will help improve yield and profitability for farmers.

Because of the problems outlined above the work reported in this thesis was conducted with the following objectives:

1. Determine the optimum level of *Rhizobium* inoculant application.

2. Determine the optimum sowing date for chickpeas under Canterbury conditions.

3. Investigate the effect of combined nitrogen and water stress on the nodulation and nitrogen fixation of chickpeas.

4. Evaluate the effects of water stress on chickpea growth in the glasshouse.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Pulses, once referred to as the poor man's meat (Smartt, 1976), are becoming increasingly important in crop production systems. They fix atmospheric nitrogen (Evans, 1982), are fodder for livestock (Saxena, 1988) and complement cereals in providing essential amino acids for human nutrition (Kay, 1979; Hulse, 1991). Chickpea (*Cicer arietinum* L.), ranks third in the area under production among the pulse crops after the common bean (*Phaseolus vulgaris* L.) and the pea (*Pisum sativum* L.) (van der Maesen, 1973; Nene and Kanwar, 1988; Zohary and Hopf, 1988). The crop is widely grown and consumed in Asia, the Middle East and several Mediterranean countries. Chickpeas have a larger concentration of calcium and iron than most other grain legumes (Smithson *et al.*, 1985). Its crude protein content, ranging between 12.4 and 31.5 per cent, is also superior in nutritional quality (Corbin *et al.*, 1977; Geervani, 1991). With few pests and diseases it is a suitable alternative rotation crop (Kay, 1979). Opportunities for export also exist to Europe, Asia and America (van Rheenen, 1991).

2.2 Origin and distribution

Although the identification of chickpea in the carbonised form is difficult (van der Maesen, 1972; Ladizinsky and Alder, 1976), the oldest chickpea find excavated from Hacilar (Turkey) has been carbon dated to 5450 BC (van der Maesen, 1984; van Rheenen, 1991). The cultivated chickpea therefore most probably originated from south eastern Turkey (van der Maesen, 1987) and was domesticated in the same region as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and pea (*Pisum sativum*) (Smartt and Hymowitz, 1985; Hopf, 1986; Zohary and Hopf, 1988). *Cicer bijugum* K.H. Rech, *C. echinospernum* P.H. Davies and *C. reticulatum* Lad., three wild species closely related to the cultivated chickpea are also found in this region (Hopf, 1986; van der Maesen, 1987).

From its origins in the fertile crescent chickpea cultivation spread into the Mediterranean countries, North Africa and Ethiopia (Kay, 1979; van Rheenem, 1991). Then eastward into India where the earliest records of chickpea dates from about 4000 BC (Smithson *et al.*, 1985). The Spanish and Portuguese introduced the crop to south and central America where it has become an important crop in Mexico, Argentina, Chile and Peru (Duke, 1981). Unlike peas and faba bean (*Vicia faba* L.) there has been limited northern penetration of chickpea into Europe possibly because of the wetter growing conditions (Smartt, 1990).

Of the two main types of chickpea cultivated, the *desi* type accounts for 85 percent of world production (Smithson *et al.*, 1985). In 1991 total chickpea production was estimated at 7.7 million tonnes from 10.7 million hectares (FAO, 1992). It is widely grown in India, the world's leading producer of chickpeas. The *kabuli* type is more predominant in West Asia and North Africa (Saxena and Goldsworthy, 1988) and is preferred in North America and Europe (Saxena, 1988; Williams *et al.*, 1991). Chickpea seed yields have remained low because of limited inputs but with proper management, yields of over 4000 kg/ha have been obtained (van der Maesen, 1972). Production is declining steadily in Europe and Central America (FAO, 1991, 1992). Its introduction into Australia, south east Asia and Canada will increase the area under production (Jodha and Subba Rao, 1987; Slinkard and Vandenberg, 1993).

2.3 Taxonomy

The cultivated chickpea (*Cicer arietinum* L.), is the type species and the sole cultigen of the genus *Cicer* in the monogeneric tribe Cicerae (Alef.) (van der Maesen, 1972, 1973; Smartt, 1990). The genus consists of 33 perennial and 9 annual species (van der Maesen *et al.*, 1988) divided into the four sections; Monocicer M.G. Povov, Chemacicer M.G. Povov, Polycicer M.G. Popov and Acanthocicer M.G. Povov (van der Maesen, 1987). *Cicer arietinum* L. belongs to the subgenus Pseodonomis M.G. Povov, section I Monocicer M.G. Povov and series Arietina Lincz. (van der Maesen, 1984; Smartt, 1990). It does not exist in the wild (Purseglove, 1968) and *C. reticulatum* Lad. has been considered as its progenitor (Hopf, 1986; van der Maesen, 1987; Smartt, 1990), wild race (Zohary and Hopf, 1988) or subspecies (Moreno and Cubero, 1978). This is because it crosses readily and produces fertile hybrids

with the cultivated chickpea (Ladizinsky and Alder, 1976; Smartt and Hymowitz, 1985).

2.4 Botanical characteristics

Chickpea is a short stature annual shrub exhibiting morphological variation in most of its traits (Ladizinsky and Alder, 1976). It may be erect or spreading (Purseglove, 1968), unbranched or highly branched (van der Maesen, 1972) and rarely growing taller than 60 cm (Kay, 1979). The two main types of chickpeas are *kabuli* and *desi* (Nene and Kanwar, 1988). The *kabuli*, having been derived from the *desi* through selection (Moreno and Cubero, 1978) are regarded as more advanced (Smartt, 1990). They have white flowers and aerial parts with no anthocyanin while *desi* has purple flowers and anthocyanin pigmentation (van Rheenen, 1991). *Kabuli* seeds are cream coloured and larger (260-640 g/1000 seeds) than the yellow to black seeds of the *desi* (< 260 g/1000 seeds) (Smartt, 1990). Other types of chickpea generally confined to India are the pea shaped and green seeded types.

Germination in chickpea is hypogeal. The root consists of a well developed tap root that extends to 1 m deep with four rows of lateral roots (van der Maesen, 1972; Kay, 1979). Leaves are imparipinnate and vary in colour from yellow-green to dark blue (Purseglove, 1968). Chickpea flowers are solitary, the calyx united and the corolla white, pink or blue (Duke, 1981). The anthers are uniform in size and versatile or alternately versatile (Kupicha, 1981). Flowers are mainly self pollinated (Nene and Kanwar, 1988) but occasional natural cross-pollination occurs (Smithson *et al.*, 1985). Pods contain 1-2 seeds and the seed coat may be smooth, rugose or granulate (Ladizinsky and Alder, 1976).

2.5 Nitrogen fixation

The excessive use of nitrogen fertilizer is causing global environmental concerns and other sources of nitrogen must be used if the increasing demand for food is to be met. Biological nitrogen fixation is a suitable alternative and has become the second most important biochemical process on earth after photosynthesis (Vance *et al.*, 1988). Most of the biologically fixed nitrogen in agriculture is derived from bacteria in symbiotic association with

legumes (Bauer, 1981). Bacteria in the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* are the major contributors (Kijne, 1992). Cyanobacteria, frankia and free living bacteria also fix a substantial amount of nitrogen (Bohlool *et al.*, 1992). When effectively nodulated, legumes can fix between 60 and 80 percent of their nitrogen requirements (Papastylianou, 1988). The nitrogen fixed by the legumes may also benefit subsequent cereal crops. In northern Syria, wheat grain yield following lentil (*Lens culinaris* medik.), faba bean or dry peas was higher than that following wheat or fallow (Saxena, 1988). The bacteria that form symbiotic association with chickpea are very specific (Corbin *et al.*, 1977). They are Hup- and do not cross inoculate with other groups except with *Sesbania bispinosa* and *S. sesban* (Gaur and Sen, 1979; Subba Rao, 1988). Sprent and Minchin (1985), classify this bacteria as fast growing but Ruiz-argueso *et al.* (1988), have concluded that they are intermediate between fast and slow growing groups. More recently they have been placed in the genera *Bradyrhizobium* and are now known as *Bradyrhizobium* sp. (Chickpea) (Rupela and Beck, 1990). *Rhizobium* will be adopted in this thesis as the name is still widely used.

2.5.1 Nodulation

2.5.1.1 Infection

Rhizobia can be found living saprophytically in most soils. However, their numbers increase and may reach 10 to 200 fold higher in the presence of a germinating legume seedling (Pueppke, 1986). The *Rhizobium*-legume recognition mechanism is not clearly understood but host plant lectins are thought to be involved (Sprent and Minchin, 1985). Molecules in the plant root exudates (e.g. flavonoids and isoflavonoids) may also stimulate the activity of bacterial nodulation genes (Giller and Wilson, 1991). It is at this stage that the *Rhizobium*-legume specificity is expressed (Dazzo and Truchet, 1984). The cells most susceptible to infection are those located just above the region of root elongation (Bhuvaneswari *et al.*, 1980). Rhizobia generally enters the plant through the root hairs except in genera like *Arachis* and *Stylosanthes* where this occurs through the sites of lateral root emergence (Kijne, 1992). The bacteria penetrate the cell wall by exerting mechanical pressure or releasing enzymes that degrade the cell wall (Pueppke, 1986).

2.5.1.2 Nodule initiation and development

Rhizobium-legume specificity continues through nodule development. Rhizobia release molecules (eg. NodRm-1) that induce cell divisions in the meristematic tissue of legume roots to produce nodules (Vance *et al.*, 1988; Kijne, 1992). Nitrogenase is then synthesized and bacterial enzymes that normally assimilate ammonia are repressed before nitrogen fixation begins (Sprent and Minchin, 1985). Nodules actively fix nitrogen for several weeks then senesce. Nodule initiation and development in legumes are influenced considerably by soil temperature. Optimum temperature for nodule initiation ranges between 18 °C and 24 °C (Kumarasinghe and Nutman, 1979). Higher temperatures progressively delay nodulation but less strongly than falling temperatures.

2.5.2 Amount of nitrogen fixed

2.5.2.1 Methods of estimating nitrogen fixed

It is necessary to quantify the amount of nitrogen fixed by the *Rhizobium*-legume symbiosis to determine its contribution to an agricultural system. An accurate measurement of the nitrogen fixed in the field is difficult. There are many measurement techniques each with specific advantages and disadvantages (Peoples and Herridge, 1990). Total nitrogen accumulated is the simplest estimate of the amount of nitrogen fixed. However it overestimates nitrogen fixation because of the assumption that the crop derives all this nitrogen from biological nitrogen fixation (LaRue and Patterson, 1981).

More reliable fixation estimates can be obtained if the amount of nitrogen derived from the soil is known. This can be determined by growing a nonlegume, nonnodulating isoline or uninoculated legume under identical conditions as the nitrogen fixing legume (Peoples and Herridge, 1990). To determine the amount of nitrogen fixed, the total nitrogen content of the nonfixing crop is subtracted from the total nitrogen content of the fixing legume (LaRue and Patterson, 1981). Estimates of nitrogen fixed determined by this method (difference method) have been variable. This may be a reflection of the choice of nonfixing control crop or variability in environmental conditions. In a soil free of native chickpea rhizobia, 51 kg N/ha

was fixed by a chickpea crop in Syria (Cakmakci *et al.*, 1988). Using barley (*Hordeum vulgare* L.) and rye grass (*Lolium perenne* L.) as the nonfixing control crops, Papastylianou (1987) has reported that chickpea fixed 15 and 28 kg N/ha respectively. In soybean (*Glycine max* L.) the estimates have been fairly consistent. Peoples and Herridge (1990), have reported nitrogen fixed in the range between 128 and 150 kg N/ha and is consistent with the 144 to 188 kg N/ha obtained by Eaglesham *et al.* (1982). This method assumes that all the nitrogen contained in the nonfixing control is derived from the soil. Further the two crops are assumed to assimilate an equal amount of nitrogen from the soil. However, the nitrogen fixing plant is likely to absorb less nitrogen from the soil (Giller and Wilson, 1991).

The acetylene reduction assay has been instrumental in the proliferation of nitrogen fixation research (Turner and Gibson, 1980). This assay involves the incubation of detached nodules or nodulated root pieces with 10% acetylene in a closed container of known volume (Peoples and Herridge, 1990). Gas chromatography is then used to measure the concentration of accumulated ethylene (Hardy *et al.*, 1973). Although it is assumed that the assay procedure does not in itself affect the rate of activity, observations have shown that nitrogenase activity in many legumes declines rapidly in the presence of acetylene (Minchin *et al.*, 1983). A ratio of three to one is often used for acetylene reduced to atmospheric nitrogen fixed (van Kessel and Burris, 1983). In those species where hydrogen is evolved during nitrogen fixation, the nitrogenase activity is reduced (Hudd *et al.*, 1980). The assay is therefore inaccurate (Witty and Minchin, 1988) and may under estimate nitrogen fixed by up to 50 percent (Ledgard and Steele, 1992). Because of variations in light intensity, temperature and moisture levels, it is also difficult to integrate a series of short term assays over time (Turner and Gibson, 1980).

The ^{15}N isotope methods, based on a difference in the ^{15}N abundance between atmospheric nitrogen and soil nitrogen provide the best estimates of the amount of nitrogen fixed (Giller and Wilson, 1991). Often differences in natural ^{15}N abundance are too small and enrichment of the soil with ^{15}N labelled fertilizer improves the method (Duc *et al.*, 1988). Since the ^{15}N enriched nitrogen taken up from the soil is diluted by nitrogen of much lower ^{15}N abundance fixed from the atmosphere (Norhayati *et al.*, 1988), the extent to which the soil $^{15}N/^{14}N$ ratio is diluted gives an indication of the magnitude or efficiency of nitrogen fixation (Danso, 1988). A reference crop which does not fix nitrogen is used to measure the ^{15}N enrichment of the available soil nitrogen (Giller and Wilson, 1991). A major assumption of the ^{15}N

enrichment technique is that the legume and the reference crop absorb the same amounts of nitrogen from the added ^{15}N and from the soil (de Freitas *et al.*, 1984). Other ^{15}N isotope based techniques include the A-value modification and the ^{15}N natural abundance methods (Witty *et al.*, 1988).

2.5.2.2 Amount fixed

The *Rhizobium*-chickpea symbiosis fixes different amounts of nitrogen in different environments. Data on the amount of nitrogen fixed by chickpea in Canterbury is not available but nitrogen balance estimates show that lupins (*Lupinus angustifolius* L.) and peas fixed 183 and 75 kg N/ha respectively (Rhodes, 1980; Askin, 1983). Other reports from throughout New Zealand have shown that white clover (*Trifolium repens* L.) and rye grass mixtures can fix between 92 and 392 kg N/ha with a mean of 184 kg N/ha (Hoglund *et al.*, 1979). These studies have also suggested that seasonal and annual variations exist in the amount of nitrogen fixed by the legume-grass mixtures. On average less nitrogen is fixed in winter with mid winter fixation rates ranging between 0.2 and 0.38 kg N/ha/day (Clark *et al.*, 1979; Rumball, 1979). Seasonal nitrogen fixation rates may therefore be controlled by temperature in spring and autumn and drought in summer (Crush, 1979).

Estimates using the ^{15}N isotope techniques have shown that a chickpea crop fixed 71 kg N/ha in Cyprus (Papastylianou, 1988). This value is similar to the 75 kg N/ha (^{15}N isotope technique) obtained in a rainfed crop at Tel Hadya in northern Syria (Saxena, 1988). Rennie and Dubetz (1986), have recorded 84 kg N/ha (^{15}N isotope technique) fixed by an irrigated crop in Canada while in Australia 87 kg N/ha has been reported (Peoples and Craswell, 1992). These estimates do not compare favourably with those for other grain legumes. Cowpea fixed 92 kg N/ha (^{15}N isotope technique) (Awonaike *et al.*, 1990), and soybean 189 kg N/ha (N difference method) (Piha and Munns, 1987).

2.5.3 Factors affecting nitrogen fixation

The performance of the *Rhizobium*-legume symbiosis is determined by the host, rhizobia, their interaction and the environment. Environmental stresses can reduce rhizobial populations in the soil, disrupt infection and nodule development and the fixation of nitrogen (Sprent, 1976; Giller and Wilson, 1991). Indeed the symbiosis is more sensitive to environmental stresses than the free living rhizobia or the uptake of inorganic nitrogen (Rupela and Kumar Rao, 1987; Sprent, 1992).

2.5.3.1 Water stress

In India chickpea is grown as a post rainy season crop and encounters increasing moisture deficit. Moisture stress restricts the formation, growth and function of nodules and under severe moisture stress most legumes do not nodulate (Dart *et al.*, 1975; Sprent and Zahran, 1988). Structural alterations and reduced respiration in the nodule associated with moisture stress also causes a reduction in the amount of nitrogen fixed (Engin and Sprent, 1973). This may be further aggravated by a reduction in the host photosynthetic capacity (Sprent, 1972a; Sprent and Zahran, 1988). Therefore under conditions of water stress the amount of nitrogen fixed will depend on soil moisture (Rupela and Beck, 1990). Recovery from moisture stress depends on the severity and duration of stress and the type of nodule present. Irrigation in chickpea stimulates nitrogen fixation because of supplemental growth following irrigation and prolonged fixation which may extend into pod fill (Nambiar *et al.*, 1988; Wery *et al.*, 1988).

2.5.3.2 Temperature

The survival and multiplication of bacteria in the soil are influenced by temperature. High soil temperatures are detrimental and their effects are more pronounced in soil low in organic matter and clay (Dart *et al.*, 1975). However, legumes nodulate more slowly when temperatures are low (Corbin *et al.*, 1977). In chickpea, temperatures lower than 15 °C are sub-optimal for nitrogen fixation (Rupela and Beck, 1990). Coupled with a delay in plant growth under such conditions, the amount of nitrogen fixed is reduced. Chickpea nitrogenase

enzyme can fix nitrogen over the temperature range from 6 °C to 40 °C (Dart *et al.*, 1976), but optimal temperatures lie between 18 °C and 22 °C (Smithson *et al.*, 1985). Above 30 °C, nitrogen fixation is severely reduced because of nodule senescence (Nambiar *et al.*, 1988).

2.5.3.3 Inoculation

The efficiency of a strain in forming nodules can be lost if it is combined with a less effective strain (Islam, 1984; Somesagaran *et al.*, 1988). Desi chickpea fixed 38 percent less nitrogen when inoculated with several strains compared to a single strain (Bohlool *et al.*, 1988). Inoculation with a suitable strain is therefore important for efficient nitrogen fixation because it is difficult to replace an ineffective strain once it is established in the soil (Dart *et al.*, 1975).

2.5.3.4 Rhizobial population

Although the recommended rate of inoculum is 8-50 x 10^4 cells of rhizobia per seed for crop species (Brockwell *et al.*, 1980), inoculant rhizobia can only nodulate successfully in soils that have low populations of competing indigenous rhizobia. Large numbers of indigenous rhizobia generally outcompete introduced inoculant strains for nodule occupancy (Schmidt, 1988). This is because of their selection over time and better adaptation to environmental stresses (Bohlool *et al.*, 1988). Indigenous rhizobia are usually less effective at fixing nitrogen. In Cyprus, chickpea fixed only 25 kg N/ha without inoculation (Papastylianou, 1988). A strain selected for inoculation should therefore fix nitrogen efficiently and compete effectively for nodule occupancy in the presence of native rhizobial strains (Cleyet-marel, 1988).

2.5.3.5 Combined nitrogen

Nitrogen deficiency in legumes before sufficient nitrogen is fixed by the symbiosis is common (Sprent and Minchin, 1983; Abdel-Ghaffar, 1988). Additional nitrogen fertilizer is therefore

occasionally added to alleviate the nitrogen stress and optimize yields (Rupela and Dart, 1980). However, large amounts of inorganic nitrogen in the rhizosphere generally inhibit nitrogen fixation (Dart *et al.*, 1975; Vance *et al.*, 1988) by limiting the development of the *Rhizobium*-legume symbiosis (Dazzo and Truchet, 1984; Kijne, 1992) and reducing nitrogen fixation in those nodules already formed (Beringer *et al.*, 1988). Extensive research into the adverse effects of combined nitrogen on nitrogen fixation (eg. Streeter, 1985a,b; Streeter, 1988), has failed to determine its cause (Minchin *et al.*, 1986; Wasfi and Prioul, 1986). A number of hypotheses have been put forward to explain this phenomenon but only two have received considerable support. Oghoghorie and Pate (1971), suggest that decreased carbohydrate availability resulting from the reduction and assimilation of combined nitrogen could limit nitrogenase activity, while Arp and Zumft (1983) implicate the products of nitrate reduction; principally nitrite, as it binds and inhibits nitrogenase and leghaemoglobin (Becana and Sprent, 1987).

Because of the inadequacies of these two hypotheses (Streeter, 1981, 1985a,b; Becana and Sprent, 1987) other possibilities have been considered. It now appears that increased resistance to oxygen diffusion into the bacteroids may play an important role in the reduction of nitrogen fixation in the presence of combined nitrogen (Sprent *et al.*, 1987; Minchin *et al.*, 1988). Work with supernodulating nitrate tolerant symbiotic mutants in soybean has provided little insights into the regulation of nitrogen fixation by nitrate because the profuse nodulation observed in these plants arises from a mutational alteration in the autoregulation of nodule development (Delves *et al.*, 1986).

2.5.3.6 Nutrient deficiencies

Nutrient deficiencies may act directly on nodule activity or indirectly through the host plant (Sprent and Minchin, 1983). Most legumes have a high requirement for phosphorus and its deficiency most limits nitrogen fixation (Graham, 1981). Molybdenum is an important constituent of the nitrogenase enzyme and a deficiency may result in the initiation of many nodules that do not fix nitrogen (Abdel-Ghaffar, 1988). Multiplication of rhizobia in the rhizosphere is limited by low calcium while insufficient boron causes the formation of necrotic nodules that do not fix nitrogen (O'Hara *et al.*, 1988).

2.6 Growth and dry matter accumulation

Growth occurs because of both cell division and expansion (Fischer, 1984). It is subject to various environmental controls that affect its rate and duration. The rate and duration of growth are usually inversely related, therefore increasing temperature increases the rate of growth and reduces the duration. Under tropical growing conditions the duration of growth to 95% final pod weight in cowpea was 35 days at 28.1 °C but 60 days at 24.8 °C (Littleton *et al.*, 1979a). Growth rate is also accelerated by elevated carbon dioxide concentrations due to increased supply of carbohydrates (Terry *et al.*, 1983). Water stress by controlling nutrient uptake, carbohydrate and protein metabolism (Slayter, 1973) controls growth at the cellular level (McIntyre, 1987).

2.6.1 Seasonal dry matter accumulation

As with many crop species (eg. Herdina and Silsbury, 1990), dry matter accumulation in chickpeas follows a sigmoid growth curve. It is slow during early vegetative growth then accelerates after flowering before declining during fruit set (Khanna-Chopra and Sinha, 1987). During the period of slow growth, the crop accumulates about 2.0 g dry matter/m^2 per day. This rises to between 8 and 14 g dry matter/m^2 per day during the linear phase of growth (Saxena *et al.*, 1983). Under Canterbury conditions McKenzie and Hill (1994), have obtained maximum growth rates of between 9 and 13.1 g dry matter/m^2 per day. Partitioning of dry matter during early vegetative growth favours the roots, nodules, leaves and stems (Siddique and Sedgley, 1986). Singh (1991) reported that 48 to 51 per cent of the total above ground dry matter produced before pods were initiated was allocated to the leaves. Because of its indeterminate nature dry matter accumulation in the vegetative structures of chickpea continues into the reproductive phase (Saxena and Sheldrake, 1980). Competition between these two sinks then determines the partitioning of dry matter produced thereafter (Goldsworthy, 1984).

Both nitrogen nutrition and water availability affect partitioning of dry matter during growth. Rawsthorne *et al.* (1985a) observed that nitrate fed plants partitioned more dry matter into the branches and leaves during early vegetative growth compared to those dependant on nitrogen fixation. Water stress during reproductive growth may result in the allocation of

more dry matter into the pods and the seeds (Singh, 1991). After anthesis, proportionately more dry matter and nitrogen is partitioned into pod wall development. This nitrogen is stored temporarily in the pod and later incorporated into the seed (Farrington *et al.*, 1977). Seed growth then accounts for almost all the changes in dry weight (Summerfield *et al.*, 1980). In chickpea up to 60% of all the dry matter accumulated after anthesis is allocated into the seed (Khanna-Chopra and Sinha, 1987). While current photosynthate contributes the largest proportion of the dry matter for seed filling, between 15 and 20 percent may be derived from assimilate translocated from stems and leaves (Saxena *et al.*, 1983; Singh, 1991).

2.6.2 Total dry matter accumulation

The photosynthetic system is assembled into a physiologically functional unit in the leaves. This enables the leaves to intercept radiant energy and absorb carbon-dioxide necessary for photosynthesis (Leech and Baker, 1983). The expanding leaf therefore provides the carbon necessary for its own growth and exports to other developing structures (Dale and Milthorpe, 1983). Cereals accumulate dry matter during their vegetative growth at rates proportional to intercepted radiation if water is non limiting (Monteith, 1977; Gallagher and Biscoe, 1978; Azam-Ali *et al.*, 1989). Further studies in chickpea and field bean have yielded similar results (Hughes and Keatinge, 1983; Keatinge and Cooper, 1984; Husain *et al.*, 1988a). The constant of proportionality is a measure of the photosynthetic efficiency of the crop (Monteith and Elston, 1983). Because the rate of conversion of intercepted radiation into dry matter is fairly constant (Hughes *et al.*, 1987), differences in total dry matter accumulated result from differences in the amount of intercepted radiation (Littleton *et al.*, 1979b) which is governed by both the rate of increase and the duration of total leaf area (Gallagher and Biscoe, 1978; Terry *et al.*, 1983).

Chickpea loses most of its leaves before final harvest and this may lead to an underestimation of the total dry matter accumulated (Saxena and Sheldrake, 1980). Khanna-Chopra and Sinha (1987), estimated that leaf fall can account for almost 20 to 30% of the loss in the total dry weight of the plant. Total dry matter accumulated varies from 1.4 t/ha to about 7 t/ha (Siddique and Sedgley, 1986), although a maximum dry matter of between 8 and 10 t/ha is possible. In Canterbury, total dry matter declined as sowing date was delayed from about 7

t/ha when sowing was done in May to 4 t/ha in a sowing in October (McKenzie and Hill, 1994). These values are much lower than the 10.7 t/ha obtained in lentil by McKenzie and Hill (1990) or the 12 t/ha in field bean reported by Husain *et al.* (1988a), both also in Canterbury.

2.6.2.1 Leaf growth

Canopy growth is dependent on the appearance and expansion of leaves (Littleton *et al.*, 1979a), but final leaf size is determined by the rate of leaf expansion (Jordan, 1983). During early leaf growth, there is little or no change in average cell volume and increase in leaf size corresponds more closely to increase in cell number (Sunderland and Brown, 1956; Dale and Milthorpe, 1983). This is consistent with the observation made by Terry (1970) in sugar beet (*Beta vulgaris* L.) where leaf growth before unfolding was primarily because of an increase in cell number with cell volume becoming increasingly important after the leaf has unfolded.

Leaf expansion rates increase linearly with temperature until an optimum temperature is reached (Squire, 1990). The decreased rate of leaf expansion at higher temperatures occurs because proportionately more cells start elongating rather than undergoing more cycles of division (Milthorpe, 1959). There is also an increase in both protein denaturation and proteolytic enzyme activity at temperatures greater than optimum (Terry *et al.*, 1983). Because cell expansion is more a result of increased water uptake, the reduction in leaf growth because of water stress is a direct consequence of the lack of turgor needed for cell expansion (Acevedo *et al.*, 1971). Cell expansion is therefore extremely sensitive to moisture stress and leaf area increase may often occur at night when water deficits are less limiting (Hsiao and Acevedo, 1974; Bunce, 1978). Water stress therefore leads to the initiation of smaller and fewer leaves (Farah, 1981). Additional nitrogen increases leaf area by increasing the number and size of cells (Terry, 1970). Generally, temperature will have its greatest influence on the duration of leaf growth unlike water and nitrogen that mainly affect the mean rate of expansion (Monteith and Elston, 1983). Since there is a progressive increase in leaf size with increase in position up to a certain point on the stem, the lower leaves are increasingly deprived of sufficient light and leaf senescence may occur even before maximum leaf area is achieved (Dale and Milthorpe, 1983).

Leaf area increase in chickpea is very slow during early vegetative growth. Khanna-Chopra and Sinha (1987) have reported a leaf area of between 70 and 80 cm^2 85 days after sowing while McKenzie and Hill (1994) have recorded an LAI of 1.35 in November from a May sowing. Favourable temperatures increase leaf area but also hasten leaf senescence. In chickpea where each subtending leaf provides most assimilate for pod development, leaf fall reduces pod formation and consequently seed yield (Pandey, 1984). Delaying the onset of senescence and lengthening the duration of growth usually increases seed yield (Littleton *et al.*, 1979b).

2.6.2.2 Nitrogen nutrition

In chickpea, about 43% of the seed nitrogen is mobilized from the vegetative structures during seed filling with the leaves and petioles contributing 74% of this amount (Summerfield *et al.*, 1980). It is no surprise therefore that the nitrogen content of the leaves has been reported to decline from 5% at the beginning of seed filling to 2% just before abscission (Khanna-Chopra and Sinha, 1987). Since the crop does not fix sufficient nitrogen for maximum growth it may be necessary to add small quantities of inorganic nitrogen (Hernandez and Hill, 1984; Wery *et al.*, 1988). Addition of inorganic nitrogen increases leaf area and dry matter accumulation which leads to the initiation of more pods/plant (Rawsthorne *et al.*, 1985a; McKenzie and Hill, 1994). Similar responses have been reported in cowpea (*Vigna unguiculata* L.) (Summerfield *et al.*, 1977) and common bean (*Phaseolus vulgaris* L.) (Awonaike *et al.*, 1980). In lentil, additional nitrogen promoted branch production (Summerfield *et al.*, 1989). Since the primary and secondary branches contribute the most yields in chickpea (Hernandez and Hill, 1983), seed yield may benefit from additional inorganic nitrogen. McKenzie and Hill (1994) have obtained seed yield increases of 17% and 43% with 50 and 100 kg N/ha respectively. However, no seed yield increases with nitrogen fertilizer application were obtained in common bean despite substantial increases in dry matter accumulation (Awonaike *et al.*, 1980).

2.6.3 Seed yield

Seed yield is the product of many processes and is influenced to a large extent by environmental factors (Jamieson and Wilson, 1982). It depends on total dry matter accumulated during the growing season but also important is the proportion of this dry matter that is partitioned into the seeds (Sheldrake and Saxena, 1979; Muchow and Charles-Edwards, 1982). Chickpea seed yield varies from one site to another and from one season to the next but in the traditional chickpea growing areas it averages about 700 kg/ha (Summerfield *et al.*, 1980; Nene, 1987). However, yields over 3000 kg/ha have been obtained with winter sowing in the Mediterranean region (Hawtin and Singh, 1984; Saxena M.C., 1984). This has been attributed to the accumulation of more total dry matter, production of more seeds/unit area and an improved harvest index (Saxena *et al.*, 1990). In Canterbury, experimental evidence indicates that up to 3.5 t/ha can be obtained when environmental conditions are favourable (McKenzie *et al.*, 1992; McKenzie and Hill, 1994).

2.6.3.1 Sowing date

Chickpea is normally sown during winter in India and during spring in the Mediterranean region and depends on residual moisture (Hawtin *et al.*, 1980; Saxena and Sheldrake, 1980; Summerfield *et al.*, 1980). It can tolerate temperatures as low as - 9.5 °C during early vegetative growth and may even remain under snow for 3 months without much damage (Duke, 1981; Kumar *et al.*, 1988). In the Mediterranean environment, conditions in winter are favourable for the development of ascochyta blight (*Ascochyta rabiei*). It is this disease and not the cold that encourages farmers to sow during spring (Keatinge and Cooper, 1983; Hawtin and Singh, 1984). With the isolation of ascochyta blight resistant cultivars, winter sowing has been done and yields between 3.5 and 4 t/ha have been achieved (Khanna-Chopra and Sinha, 1987). This yield increase occurs because of a more favourable vegetative and reproductive growth period.

The crop is therefore able to intercept more solar radiation and fix more nitrogen because of the increased photosynthetic area and reduced competition for assimilate between the pods and the nodules (Hughes *et al.*, 1987; Wery *et al.*, 1988). Rainfall is also well distributed in the

Mediterranean region during winter and the crop can use it more efficiently (Keatinge and Cooper, 1983; Huda and Virmani, 1987). Saxena *et al.* (1990) reported that in one season 4.93 and 2.80 kg seed yield was produced per mm of rain water received per hectare in winter and spring respectively. Winter chickpea can therefore yield well in regions with an annual rainfall as low as 250 mm (Hawtin and Singh, 1984). However, superior yields from winter sowing will only be attained if ascochyta blight resistance is accompanied by sound agronomic practises (Saxena M.C., 1980, 1984). Yield increases may not result from winter sowing in Canterbury because harvest index has been shown to decline with early sowings due to increased vegetative growth with no corresponding increase in seed yield (McKenzie *et al.*, 1992; McKenzie and Hill, 1994).

2.6.3.2 Yield components

Yield components in grain legumes are the number of pods per plant, the average number of seeds in each pod and the mean seed weight (Summerfield *et al.*, 1980). Development of yield components begins with the number of pods, then the seed in each pod and finally the average seed weight (Slinkard and Sinhu, 1988). Seed weight is the most stable component of yield and variations in seed yield frequently are due to variations in the number of pods per unit area (Littleton *et al.*, 1979b; Saxena and Sheldrake, 1980; Saxena, 1980; Saxena *et al.*, 1983). Negative correlations between yield components is a common phenomenon in many crops under various kinds of environmental stresses (El Nadi, 1970). Because the development of yield components are interdependent, these correlations are thought to be developmental rather than genetic (Adams, 1967). In chickpea for example, pod number and seed weight decreased linearly with increasing plant populations (Hernandez and Hill, 1983). Similar negative correlations between yield components have also been reported by Siddique and Sedgley (1986). This response being due to intraplant competition for essential nutrients, results in the maintenance of a stable yield (Adams, 1967).

Since the number of reproductive nodes greatly affects seed yield (Goldsworthy, 1984), the double podded character has a potential to increase seed yield (Hawtin *et al.*, 1980; Saxena and Sheldrake, 1980). Nene (1987), has reported a yield advantage of about 11 percent from plants exhibiting this character.

2.6.4 Harvest index

Harvest index in grain legumes is very variable (Husain *et al.*, 1988b; McKenzie and Hill, 1990) and except for soybean and groundnut is usually less than that for cereals (Lawn, 1989). In chickpea the massive leaf falls before final harvest leads to the harvest index being over estimated by about 10 per cent (Saxena and Sheldrake, 1980). It generally varies between 30 and 50 percent (Saxena N.P., 1984; Siddique and Sedgley, 1986) but a harvest index of 60 per cent has been reported by Nene (1987). Irrigation may reduce harvest index because of a response in total dry matter production than in seed yield (McKenzie and Hill, 1990). Work on chickpeas in Canterbury has indicated that nitrogen fertilizer application can increase harvest index (McKenzie *et al.*, 1992).

2.7 Conclusions

(1) Chickpeas have the potential to yield up to 3.5 to 4 t/ha in New Zealand.

(2) Dry matter production in chickpea may respond to nitrogen fertilizer application but the response of seed yield is more variable.

(3) Winter sowing can double the seed yield of chickpea in the Mediterranean region but a similar response has not been obtained in New Zealand.

(4) Nodulation with *Rhizobium* is necessary to ensure efficient nitrogen fixation. However, chickpeas appear to require additional nitrogen for maximum seed yields.

CHAPTER 3

MATERIALS AND METHODS

The data presented in this thesis are derived from three experiments; two carried out in the field and one in the glasshouse.

3.1 Experiment 1

The aims of this field experiment were to:
(i) Investigate the effect of nitrogen fertilizer application on chickpea nodulation
(ii) Determine optimum *Rhizobium* inoculant application
(iii) Determine the optimum sowing date for chickpeas in the Canterbury environment.

The experiment was carried out on a Templeton silt loam soil (NZ Soil Bureau, 1968) at the Lincoln University Henley Research Farm. The site was previously under ryegrass and standard farm practices were used to establish the crop. A Ministry of Agriculture and Fisheries soil quick test gave the following results: pH 6.1, Ca 16, P 26, Mg 24, Na 4, S 2 and plant available N was 12.8 kg N/ha. The experimental design was a split plot randomized complete block with two sowing dates, 3 July and 30 September 1992, as main plots and a factorial combination of four levels of nitrogen (0, 15, 45 and 90 kg N/ha) and three levels of *Rhizobium* inoculation (0, recommended rate (240 g/100 kg seed) and twice the recommended rate) as the 12 subplots. There were three replicates and each plot was 2.1 m x 10 m.

Locally obtained *kabuli* chickpeas (unnamed commercial cultivar) with a 1,000 seed weight of 450 g and a germination of approximately 60% was used. Seed was treated with the fungicide Apron 70 SD (a.i. metalaxyl 350 g/kg and captan 350 g/kg) at a rate of 200 g (dissolved in 500 ml of water) per 100 kg seed. *Rhizobium cicerri*, strain CC1192 obtained from the Coated Seed Company, Christchurch was added in the required amount to 100 ml of water to form a thick slurry and then mixed thoroughly with the chickpea seed. The inoculated seed was left over night to dry before being drilled the following day. Calcium

ammonium nitrate (27% N) at the required rate was broadcast onto the plots by hand before drilling. The seed was drilled on the two sowing dates using an Öyjord cone seeder to give a plant population of about 45 plants/m^2. Weed control was achieved by a pre-sowing application of cyanazine at 1.7 kg/ha in 320 l of water/ha. Further weed control was by hand.

3.1.1 Sampling

3.1.1.1 Nodule yield

Root samples for nodule counts were taken three times during the growing season. Three plants were selected at random from the 0 and 90 kg N/ha plots and their shoots were severed and roots recovered for nodule counting. This was achieved by forcing a cylinder of diameter 10.5 cm and height 20 cm, centrally positioned above the tap root to a depth of 15 cm and recovering the soil and roots it contained. The 15 cm depth was considered appropriate because Sheldrake and Saxena (1979) have reported that most of the nodules in chickpea are found in the top 0 to 15 cm of the soil. Roots and shoots were then oven dried to constant weight and their dry weight taken.

3.1.1.2 Dry matter

Growth was followed by sampling above ground dry matter fortnightly. Cuts from 0.2 m^2 quadrats were selected at random and harvested from each plot and oven dried to constant weight at 70 °C. Mean dry matter accumulation for each of the four nitrogen treatments during the growing season was described using a sigmoid growth function. This involved fitting sigmoidal growth curves of the form described by Gallagher and Robson (1984) using the Maximum Likelihood Programme (MLP) (Ross *et al.*, 1987). Each generalized logistic curve fitted to the dry matter accumulated by the July sowing was of the form:

$$Y = C/(1 + T \exp(-b(x-m)))^{1/T} \qquad \text{(Equation 3.1)}$$

Where C is the final above ground dry matter and T, b and m are constants. From the values

of C, T, b and m, the weighted mean absolute growth rate (WMAGR), maximum crop growth rate (C_m) and duration of growth (DUR) were derived as follows:

$$WMAGR = bC/2(T+2) \quad \text{(Equation 3.2)}$$

$$DUR = 2(T+2)/b \quad \text{(Equation 3.3)}$$

$$C_m = bC/(T+1)^{((T+1)/T)} \quad \text{(Equation 3.4)}$$

A logistic curve of the form shown below was fitted into the mean dry matter accumulated by the September sowing.

$$Y = C/(1 + \exp(-b(x-m))) \quad \text{(Equation 3.5)}$$

For this curve, WMAGR = bC/6, C_m = the slope at the point of inflection, and DUR was calculated from the relationship WMAGR x DUR = maximum total yield.
Because all the curves were fitted to the dry matter values which were the means of the three replicates, statistical analysis of the derived growth variates is not possible.

3.1.2 Field measurements

3.1.2.1 Leaf area index

Leaf area index was measured on 24/11/92, 24/12/92 and 26/1/93 using a plant canopy analyzer (LICOR LAI 2000).

3.1.2.2 Light interception

Solar radiation intercepted was measured every fortnight using two miniature tube solarimeters, model TSM (Delta-T devices, Cambridge, England). Both solarimeters were 0.32 m long and one was placed above and the other below the canopy. A relative sensitivity check was done for 30 seconds at the start of the measurements and then after every six plots. The counts on both channels were normally within ± 5% of each other. If this was not so,

the solarimeters were cleaned before a second relative sensitivity check was done. Solarimeter output was integrated for 20 seconds using a two-channel integrator (Systel Engineering Ltd, Christchurch, New Zealand). Four sets of readings per plot were taken. All measurements were made between 1100 and 1500 hours New Zealand Standard Time because at this time the angle of the direct solar beam was not important (Szeicz, 1974a).

From the values of the relative sensitivity (R_s), number of counts recorded by the above (C_o) and below (C_b) canopy solarimeters, the fraction of light transmitted through the canopy (T) was found following the method of Gallagher (pers. comm.).

$$T = R_s \times C_b/C_o \qquad \text{(Equation 3.6)}$$

If $\tau_p = 0.1$ and $\tau_t = 0.25$, it can be shown that T_p reduces to $T_t^{1.2}$ and further
$F_p = (1-T_t^{1.2})/1.11$ (see Gallagher and Biscoe, 1978).
Where τ_p is transmission coefficient for PAR, τ_t transmission coefficient for total solar radiation, T_p is the fraction of PAR transmitted through the canopy and F_p is the fraction of radiation intercepted.

3.1.3 Yield and yield components

At final harvest total above ground dry matter and seed yield was estimated from an area of 2.0 m^2 consisting of two 1.0 m^2 cuts taken from the central part of each plot. Five plants selected at random from the central 3 rows of each plot were taken for yield component determination.

3.1.4 Total nitrogen determination

For nitrogen analysis, seed and straw from the three replicates of each treatment were bulked together separately. A small sample of each was ground to pass through a 1mm sieve and percent nitrogen determined using an automatic nitrogen analyzer (Kjeltec auto sampler system 1035 analyzer).

3.2 Experiment 2

This glasshouse trial was designed to investigate the effect of combined nitrogen, inoculation and water stress on nodulation, nitrogen fixation and growth of chickpeas. It was a 3 x 2 x 2 randomised complete block factorial design with eighteen replicates to allow 3 sequential harvests, each of 6 replicates. The treatments were 3 levels of supplemental watering (1/3 field capacity, 2/3 field capacity and field capacity), 2 rates of nitrogen (0 and 90 kg N/ha) and 2 rates of inoculation (0 and the recommended rate).

Kabuli chickpeas with 1000 seed weight of 380 g were washed with Janola (a.i. 5% sodium hypochlorite) and rinsed several times with distilled water. The seed was then treated with the fungicide Apron 70 SD (a.i. metalaxyl 350 g/kg and captan 350 g/kg) and germinated in petri dishes kept at room temperature. Seventy per cent germination was achieved within five days. To avoid contamination with inoculum, the germinating seed not requiring inoculation was sown first. Two pregerminated seedlings were sown on 28/5/93 into each 15 cm pot filled with nitrogen free potting mix. The remaining seed was then inoculated with *Rhizobium cicerri* strain CC1192 and sown immediately. A light irrigation was provided to aid emergence. All seedlings emerged within 7 days. Thinning was done to leave one healthy plant per pot. Calcium ammonium nitrate (27% N), enough for all the pots to be fertilized was dissolved in 5.4 litres of water and each pot received 50 ml of the solution on 4/6/93. The unfertilized plots each received 50 ml of water. The pots were watered twice weekly to replace water lost through evapotranspiration. The temperature in the glasshouse was maintained at approximately 25 °C.

3.2.1 Sampling and measurements

Harvests were taken 30, 45 and 60 days after sowing. The number of branches and leaves was counted and the green area determined using a leaf area meter (CI-201 area meter, CID, Inc.). The root system was recovered and washed thoroughly to remove all the potting material and the number of nodules counted. The shoot and root samples were oven dried to constant weight and dry weights recorded.

3.2.2 Total nitrogen determination

Root and shoot material from the 6 replicates of each treatment in the last harvest were bulked together separately. A sample of each was ground to pass a 1mm sieve and per cent nitrogen determined as in experiment 1.

3.3 Experiment 3

This short field experiment investigated more thoroughly the effect of sowing date on nodulation. It was carried out on the same site as experiment 1 but in a different area. A Ministry of Agriculture and Fisheries soil quick test gave the following results: pH 6.0, Ca 11, K 14, P 18, Mg 23, Na 7 and S 10. The experiment was a split plot randomized complete block design with 3 sowing dates (9 July, 9 August and 14 September 1993) as main plots and 2 rates of nitrogen (0 and 90 kg N/ha) in the subplots. The treatments were replicated four times. Seed was effectively inoculated with *Rhizobium cicerri* (double the recommended rate) and sown on the three dates to achieve a population of 45 plants/m^2. Fertilizer application, weed control, plot size and sampling procedures were as described in experiment 1. However only nodules were sampled in this experiment.

3.4 Data analysis

To determine the effects of the various treatments, an analysis of variance was performed using the Genstat statistical package (Rothamsted, 1980).

CHAPTER 4

WEATHER DATA

4.1 1992/93 season

Mean daily soil temperatures (10 cm) during the 1992/93 growing season ranged between 2.2 °C and 13.6 °C and were usually lower than the long term means. The early part of the season (July to September) was exceptionally cold with soil temperatures being 38 to 45 percent lower than long term means (Table 4.1). With the exception of July and November, minimum daily temperatures were lower than long term means. Monthly mean total rainfall between August and October was higher than long term means. In August alone almost three times the average rainfall was received. Mean daily solar radiation receipts increased as the growing season progressed but until November were between 1 and 27% lower than the recorded long term means.

Table 4.1. Meteorological data reported from Broadfield meteorological station in Lincoln, Canterbury during the 1992/1993 season with the long term means in parentheses.

Month	Max. daily temp. (°C)	Min. daily temp. (°C)	Mean daily soil temp. (10 cm) (°C)	Solar rad. ($MJ/m^2/d$)	Monthly total rainfall (mm)
Jul-92	11.3 (10.1)	2.5 (1.4)	2.2 (4.0)	4.8 (6.4)	63.0 (68)
Aug.	9.8 (11.4)	2.2 (2.7)	3.2 (5.2)	6.9 (9.4)	166.8 (62)
Sep.	10.6 (14.2)	3.3 (4.6)	4.6 (7.6)	10.8 (10.8)	74.2 (47)
Oct.	14.5 (16.8)	6.6 (6.7)	8.0 (10.8)	16.4 (17.9)	81.2 (49)
Nov.	18.5 (18.8)	9.2 (8.1)	11.6 (13.5)	20.5 (20.8)	45.8 (53)
Dec.	18.2 (20.4)	9.6 (10.4)	12.8 (16.0)	22.1 (21.3)	55.4 (57)
Jan-93	21.0 (21.3)	10.0 (11.5)	13.6 (17.3)	23.1 (21.3)	61.4 (60)
Feb.	20.2 (20.9)	10.1 (11.4)	13.4 (16.5)	20.2 (19.5)	44.0 (54)

Long term means: Mean daily maximum and minimum temperatures and solar radiation, 1975-1983; mean daily soil temperatures (10 cm), 1976-1986 and total monthly rainfall, 1930-1981.

4.2 1993 season

This season was drier than the previous one. In three out of the five months during which the experiment was conducted, monthly total rainfall was below the long term means. July, August and October were extremely dry with rainfall being only 20, 27 and 18% of the long term mean respectively (Table 4.2). Maximum temperature was on average higher and solar radiation receipts were about equal to long term means. Soil temperatures ranged between 2.4 °C and 11.6 °C and were either lower or slightly higher than average.

Table 4.2. Meteorological data reported from Broadfield meteorological station in Lincoln, Canterbury during part of the 1993 season with the long term means in parentheses.

Month	Max. daily temp. (°C)	Min. daily temp. (°C)	Mean daily soil temp. (10cm) (°C)	Solar rad. ($MJ/m^2/d$)	Monthly total rainfall (mm)
Jul-93	11.4 (10.1)	0.6 (1.4)	2.4 (4.0)	5.6 (6.4)	13.8 (68)
Aug.	11.2 (11.4)	0.6 (2.7)	5.7 (5.2)	9.1 (9.4)	16.8 (62)
Sep.	12.9 (14.2)	4.0 (4.6)	6.6 (7.6)	12.3 (13.4)	133.0 (47)
Oct.	18.6 (16.8)	6.9 (6.7)	10.9 (10.8)	18.3 (17.9)	9.0 (49)
Nov.	16.0 (18.8)	6.4 (8.1)	11.6 (13.5)	19.4 (20.8)	90.2 (53)

Long term means: Mean daily maximum and minimum temperatures and solar radiation, 1975-1983; mean daily soil temperature (10 cm), 1976-1986 and monthly total rainfall 1930-1981.

CHAPTER 5

RESULTS AND DISCUSSION - NODULATION AND EARLY GROWTH

5.1 Nodulation - field experiment

5.1.1 Inoculation

Inoculation significantly ($p<0.05$) increased the number of nodules at all sampling dates during the first growing season (Table 5.1). Nodule number per plant averaged about one in both sowings in mid November when the first nodule count was taken. Nodulation response to inoculation was influenced by both the amounts of nitrogen (N) fertilizer available and sowing date as shown by the significant ($p<0.05$) inoculation by nitrogen and inoculation by sowing date interactions. With no added N fertilizer, inoculation with twice the recommended rate of *Rhizobium* significantly ($p<0.05$) increased the number of nodules from 0.7 to 2.6 nodules/plant while there was no difference in nodule number per plant with or without inoculation in the plots receiving 90 kg N/ha (Figure 1). Uninoculated plants in the September sowing initiated only 17% as many nodules as the inoculated plants, however there was no difference in the number of nodules per plant in the July sowing between the two treatments (Figure 2).

5.1.2 Nitrogen

During the 1992/93 growing season nitrogen application significantly ($p<0.05$) reduced the number of nodules initiated early in the growing season. At the first harvest date, the addition of 90 kg N/ha reduced nodule number by 50% from 1.4 to 0.7 nodules/plant (Table 5.1). As the growing season progressed, the effects of N application diminished such that by the last harvest date both treatments had the same number of nodules. During the second growing season plants receiving 90 kg N/ha had either the same or fewer nodules per plant than the control plants (Figure 3).

Table 5.1. The effect of nitrogen application and *Rhizobium* inoculation on nodulation of chickpea sown in July and September in Canterbury during the 1992/93 season.

	Number of nodules per plant at harvest date		
Treatment	20/11/92	21/12/92	27/1/93
Nitrogen (N) kg N/ha			
0	1.4	1.6	2.0
90	0.7	1.4	2.0
SEM	0.21	0.27	0.21
Significance	*	NS	NS
Inoculation rate (I)			
No inoculation	0.7	1.0	1.3
Double recommended	1.4	2.0	2.7
SEM	0.21	0.27	0.21
Significance	*	*	*
Sowing date (S)			
July	1.0	1.6	2.3
September	1.1	1.4	1.8
SEM	0.21	0.27	0.21
Significance	NS	NS	NS
Significant interactions	Nil	IxN	SxI
CV %	67.0	61.0	37.0

At the first harvest date in the July sowing (79 DAS), the plants receiving 90 kg N/ha had 2 nodules/plant compared to the 4.75 nodules/plant when additional N was not provided. The difference between the two treatments narrowed as the growing season progressed and by 121 DAS, plants from the unfertilized plots had only 10% more nodules/plant (Figure 3a). In the August sowing, the plants given 90 kg N/ha had between 1 and 30% fewer nodules/plant (Figure 3b). There was less variation in the number of nodules/plant between the two treatments in the September sowing, with the fertilized plants initiating between 12 and 18% fewer nodules/plant at 41 and 54 DAS respectively (Figure 3c). However at 69 DAS, these plants had 6% more nodules/plant.

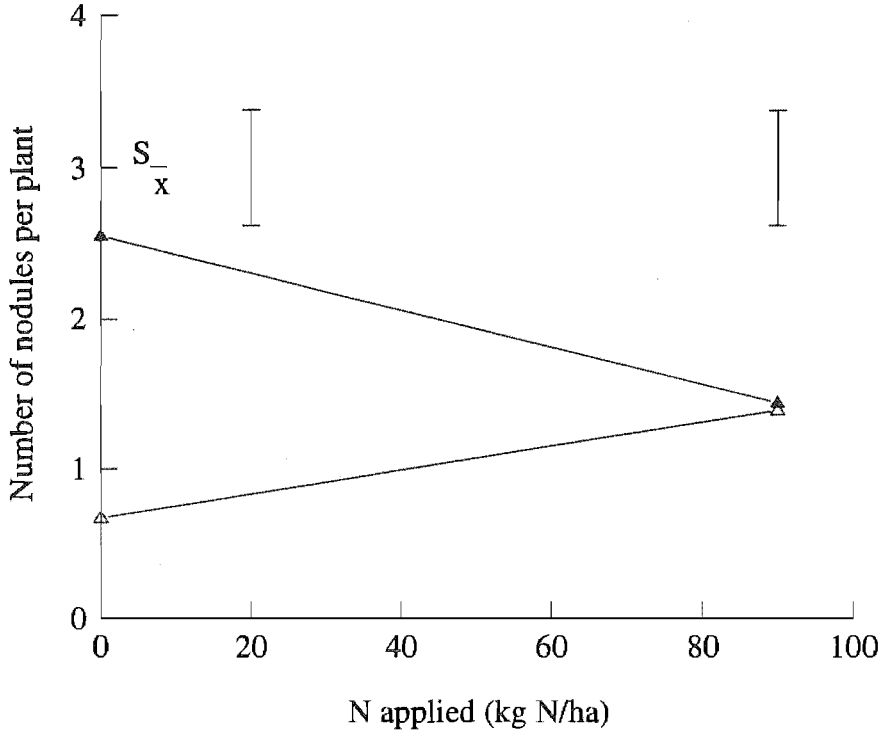

Figure 1: Nitrogen (kg N/ha) by inoculation interaction at 21/12/1992 on the number of nodules per plant in chickpea sown on two dates in Canterbury.

No inoculation (△), double recommended rate (▲).

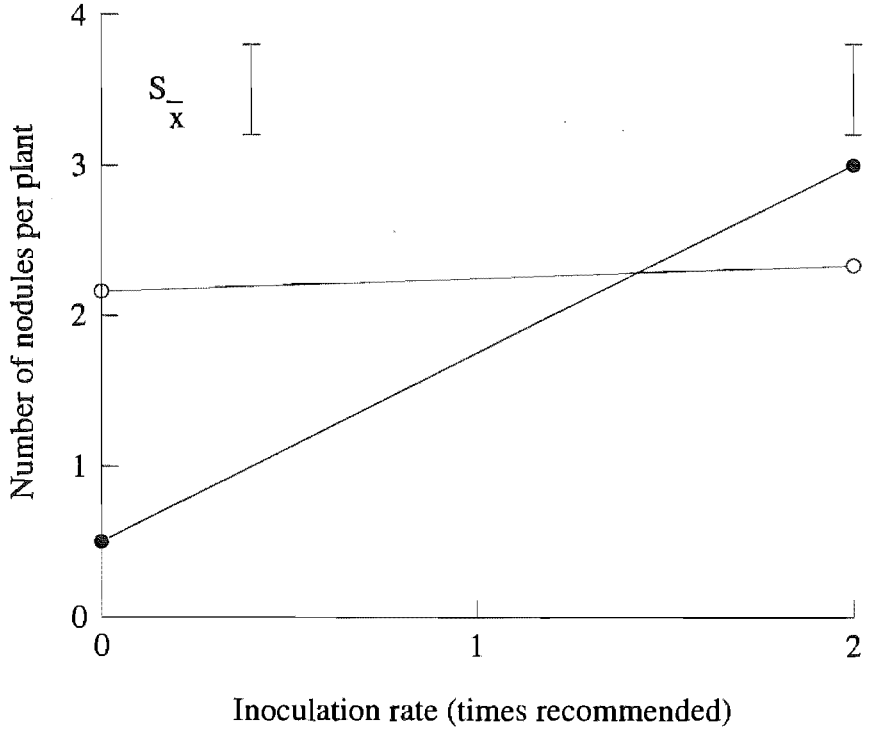

Figure 2: Inoculation by sowing date interaction at 27/1/1993 on the number of nodules per plant in chickpea sown on two dates in Canterbury.

July (○), September (●).

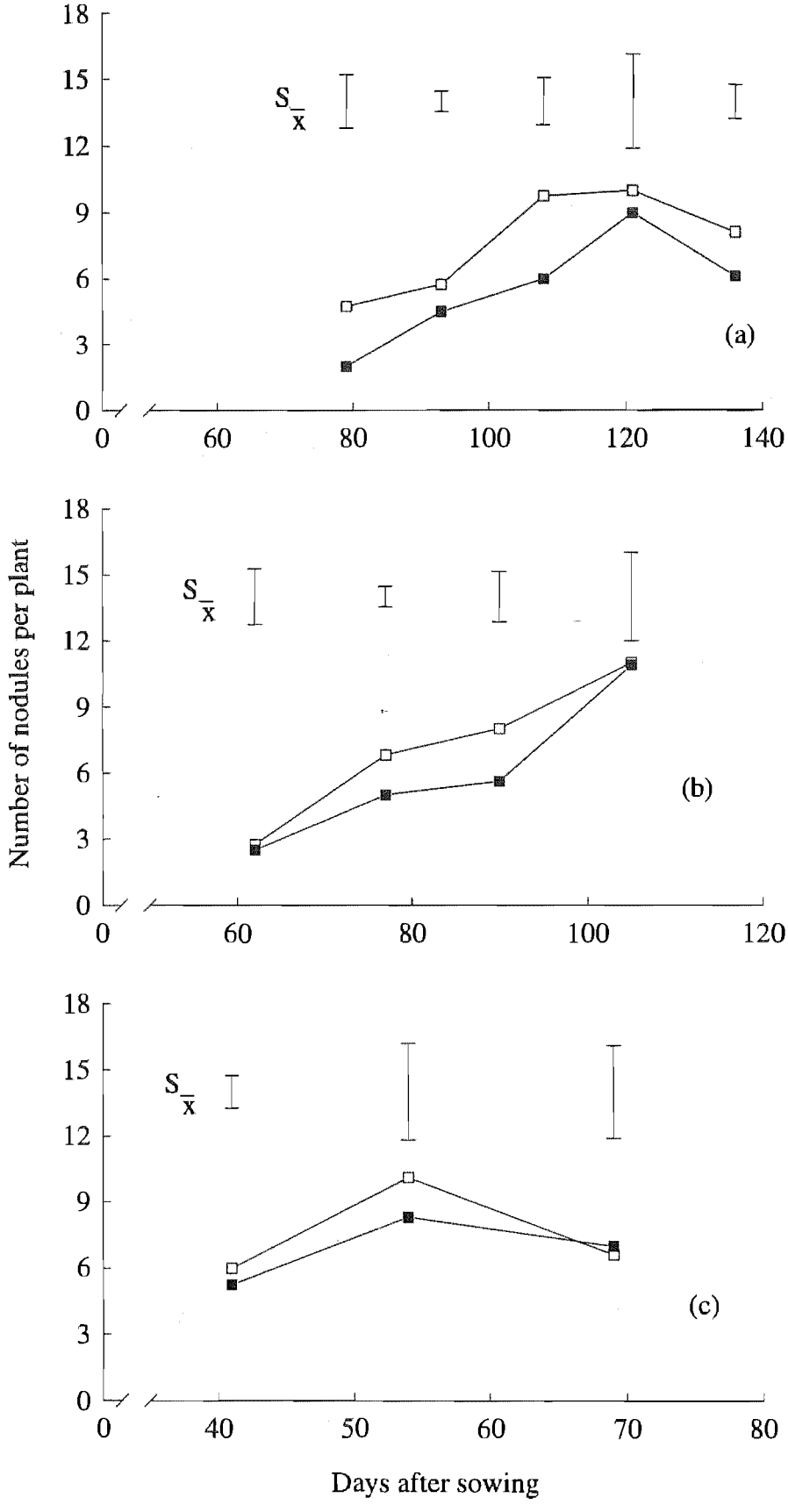

Figure 3: The effect of N application (kg N/ha) on the number of nodules per plant of (a) July (b) August and (c) September sown chickpeas in Canterbury, 1993.

0 N(□), 90 N(■).

5.1.3 Sowing date.

Throughout both growing seasons, sowing date had no significant effect on the number of nodules initiated per plant. However, during the second growing season, there were more nodules per plant at all dates than during the first growing season (Table 5.1 and Figure 4). The pattern of nodulation also varied among sowing dates in the second growing season (Figure 4). In the July sowing, nodule number/plant increased steadily from an average of 3.4 nodules/plant at 79 DAS to a peak of 9.5 nodules/plant at 121 DAS before declining after that (Figure 4). Only 3 nodules/plant were recovered from the August sowing on the first harvest date (62 DAS). This number increased continuously throughout the growing season and reached 10.9 nodules/plant at 105 DAS (Figure 4). A later harvest may have detected the eventual decline in nodule number/plant. In the September sowing, number of nodules/plant increased from 5.6 nodules/plant at 41 DAS to 9.2 nodules/plant at 54 DAS then declined to 6.8 nodules/plant at the last harvest (Figure 4).

5.2 Glasshouse experiment

5.2.1 Nodule number

Nitrogen application to potted plants significantly ($p<0.05$) reduced the number of nodules initiated throughout the experiment. At 30, 45 and 60 DAS, plants receiving additional N had 0.6, 1.1 and 1.3 nodules/plant respectively which was 16, 42 and 46% of the number of nodules on plants given no additional N (Table 5.2). The significant nitrogen by inoculation interaction at 45 DAS showed that 90 kg N/ha reduced the number of nodules initiated by 60% from 5.2 to 2.1 nodules/plant while without inoculation no nodules developed (Figure 5).

Up to 45 DAS, supplemental watering did not significantly affect the number of nodules initiated per plant. However at 60 DAS, irrigation significantly ($p<0.05$) increased it. Plants maintained at one third field capacity (1/3 FC) had 1.2 nodules/plant compared to the 2.6 nodules/plant in those maintained at field capacity (FC) (Table 5.2). The significant ($p<0.05$) inoculation by watering regime interaction at 60 DAS showed that the success of inoculation in influencing nodulation depended on water availability. Plants maintained at field capacity

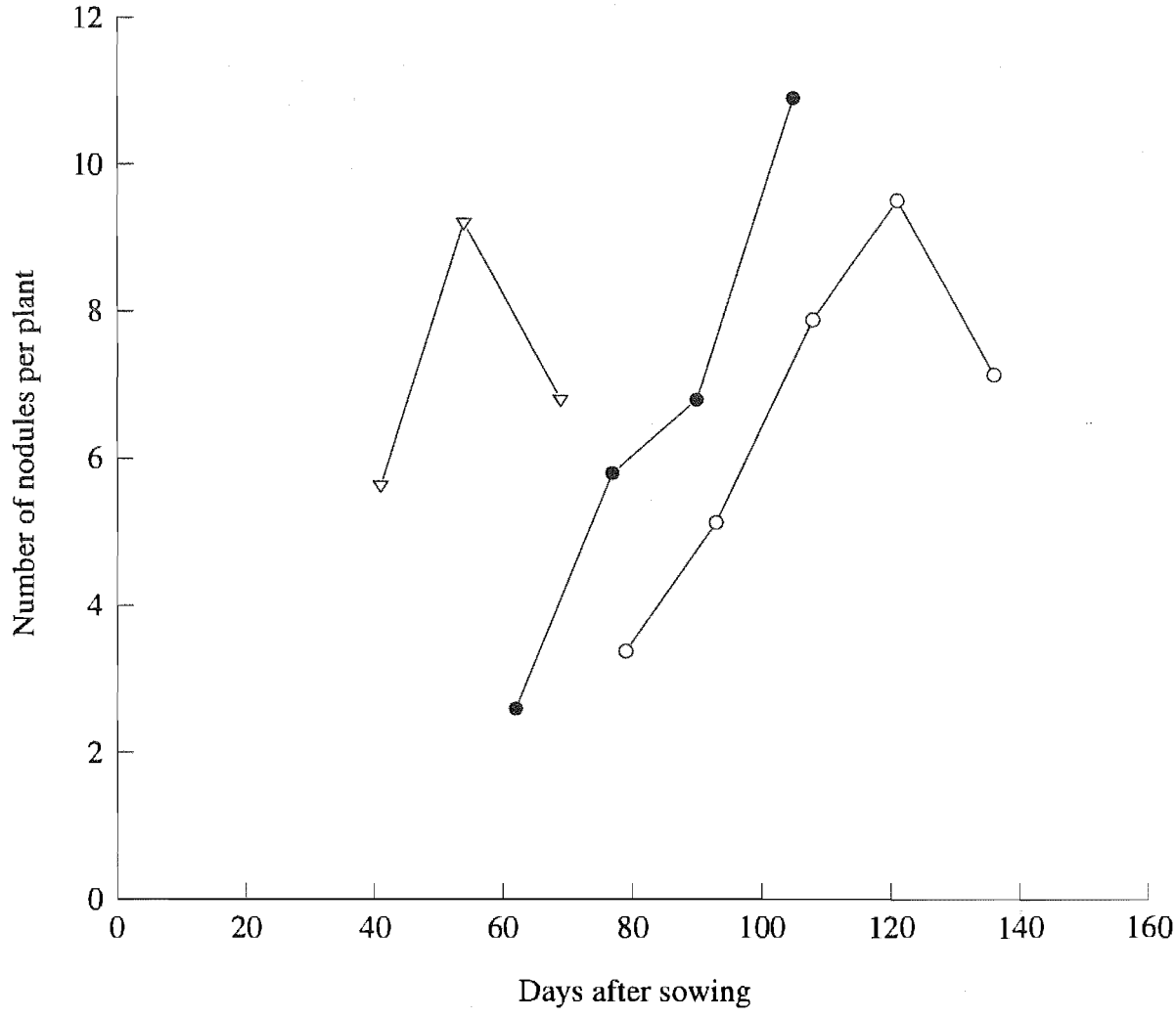

Figure 4: The effect of sowing date on the number of nodules per plant in chickpeas sown on three dates in Canterbury, 1993.

July (○), August (●), September (▽).

(See appendix 1 for $S_{\bar{x}}$).

had 5.3 nodules/plant which was 121 and 15% more nodules than in the plants maintained at 1/3 FC and 2/3 FC respectively (Figure 6).

Table 5.2. The effect of nitrogen application, watering regime and *Rhizobium* inoculation on the number of nodules per plant and cotyledon dry weight per plant of chickpeas grown in the glasshouse at 30, 45 and 60 days after sowing.

Treatment	Number of nodules per plant			Cotyledon dry weight per plant (mg)		
Nitrogen (N) kg N/ha	30	45	60	30	45	60
0	3.7	2.6	2.8	31.9	25.6	26.9
90	0.6	1.1	1.3	21.9	23.9	19.7
SEM	0.46	0.36	0.30	3.52	3.76	4.75
Significance	**	*	**	*	NS	NS
Inoculation rate (I)						
No inoculation	0.0	0.0	0.0	27.5	26.9	26.1
Recommended	4.3	3.6	4.1	26.4	22.5	20.3
SEM	0.46	0.36	0.30	3.52	3.76	4.75
Significance	**	**	**	NS	NS	NS
Watering regime (W)						
1/3 FC	2.2	1.7	1.2	31.3	37.1	30.4
2/3 FC	2.1	1.7	2.3	33.8	28.3	23.7
FC	2.2	2.1	2.6	15.8	8.7	15.4
SEM	0.56	0.44	0.37	4.31	4.6	5.82
Significance	NS	NS	*	*	**	NS
Significant interactions	Nil	NxI	IxW	Nil	NxW	Nil
CV %	128	119	89	78	91	123

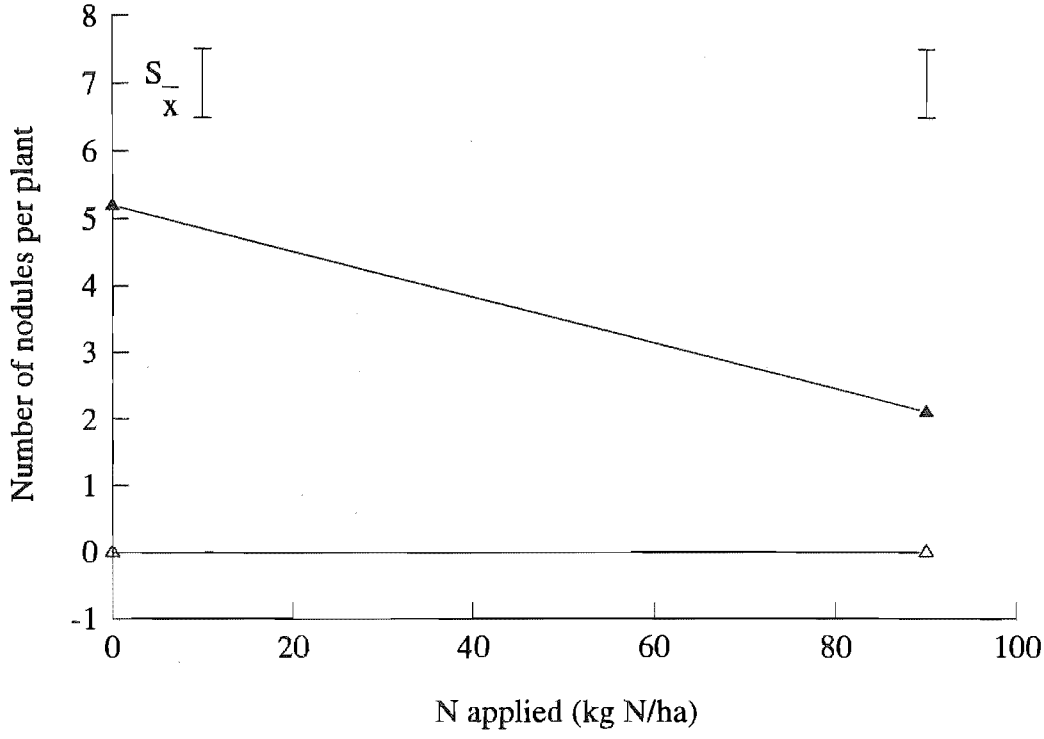

Figure 5: Inoculation by nitrogen interaction on the number of nodules per plant of glasshouse grown chickpeas at 45 DAS.

No inoculation (△), recommended rate (▲).

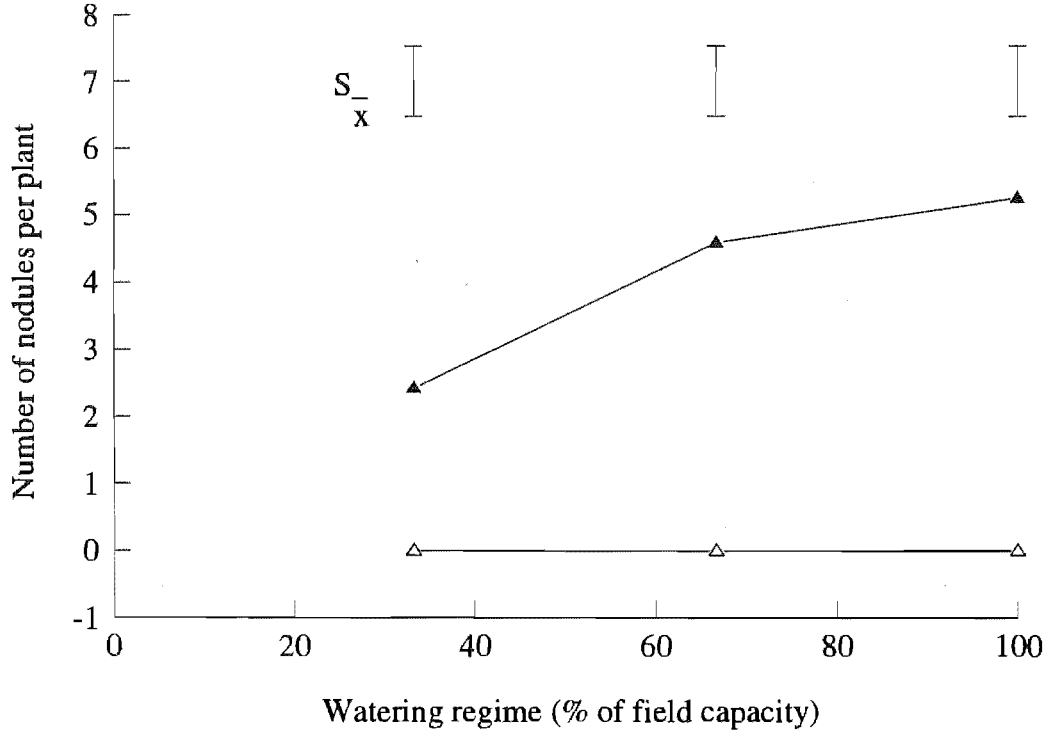

Figure 6: Inoculation by watering regime interaction on the number of nodules per plant of glasshouse grown chickpeas at 60 DAS.

No inoculation (△), recommended rate (▲).

5.2.2 Dry matter accumulation

5.2.2.1 Mobilization of seed reserves

Both nitrogen application and watering regime significantly ($p<0.05$) affected the cotyledon dry weight. At 30 DAS the cotyledons of the plants receiving 90 kg N/ha weighed 21.9 mg which was 31% less than those given no additional fertilizer N while at 30 and 45 DAS the cotyledons of the fully watered plants weighed 15.8 mg and 8.7 mg and were 50 and 77% lighter respectively, than those of the plants maintained at 1/3 FC (Table 5.2). The cotyledons of the plants maintained at 2/3 FC were also heavier than those of the fully watered plants. In the significant ($p<0.05$) nitrogen by watering regime interaction at 45 DAS seed reserves in the plants receiving 0 kg N/ha did not decline substantially until the plants were fully watered. However, with 90 kg N/ha there was a consistent decline in cotyledon dry weight as the soil water content was increased (Figure 7).

5.2.2.2 Root dry weight per plant

Average root dry weight increased throughout growth from 0.14 g/plant to 0.21 g/plant to 0.28 g/plant at 30, 45 and 60 DAS respectively. There was a significant ($p<0.05$) decrease with additional fertilizer N at 30 and 45 DAS when the plants receiving 90 kg N/ha had root dry weights that were 19 and 17% less respectively than those of the unfertilized plants (Table 5.3). Supplemental watering significantly ($p<0.05$) increased root dry weight at all harvest dates. At 30 DAS, root dry weight increased from 0.12 g/plant to 0.14 g/plant to 0.16 g/plant in watering regimes 1/3 FC, 2/3 FC and FC respectively (Table 5.3). By the last harvest (60 DAS), the difference in root dry weight had widened considerably and the fully watered plants had root dry weights that were 180 and 56% higher than those of the plants maintained at 1/3 FC and 2/3 FC respectively. The significant ($p<0.05$) inoculation by N application interaction at 60 DAS indicated that without inoculation 90 kg N/ha reduced root dry weight by 22% from 0.32 to 0.25 g/plant but with inoculation it was increased by 12% from 0.26 to 0.29 g/plant (Figure 8).

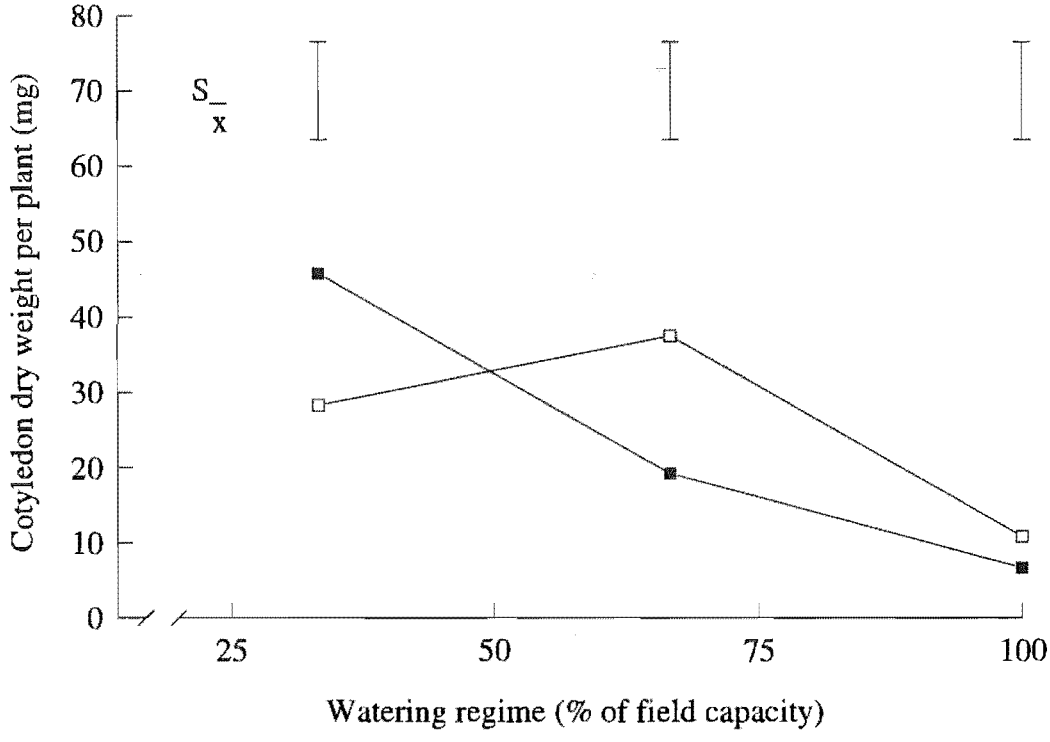

Figure 7: Nitrogen (kg N/ha) by watering regime interaction on the cotyledon dry weight per plant of glasshouse grown chickpeas at 45 DAS.

0 N (□), 90 N (■).

5.2.2.3 Branch number per plant

Nitrogen application increased branch number per plant from 3.4 to 4.1, 3.0 to 5.6 and 4.0 to 6.7, an increase of 21, 87 and 68% over the plants receiving 0 kg N/ha at 30, 45 and 60 DAS respectively (Table 5.3). At 60 DAS supplemental watering significantly ($p<0.05$) increased branch number/plant from 4 to 5 to 7 at 1/3 FC, 2/3 FC and FC in that order (Table 5.3).

Table 5.3. The effect of nitrogen application, watering regime and *Rhizobium* inoculation on the root dry weight per plant and number of branches per plant of chickpeas grown in the glasshouse at 30, 45 and 60 days after sowing.

Treatment	Root dry weight per plant (g)			Number of branches per plant		
Nitrogen (N) kg N/ha	30	45	60	30	45	60
0	0.16	0.23	0.29	3.4	3.0	4.0
90	0.13	0.19	0.27	4.1	5.6	6.7
SEM	0.007	0.009	0.014	0.23	0.37	0.67
Significance	*	*	NS	*	**	*
Inoculation rate (I)						
No inoculation	0.14	0.20	0.28	3.8	4.1	4.5
Recommended	0.14	0.22	0.28	3.6	4.5	6.2
SEM	0.007	0.009	0.014	0.23	0.37	0.67
Significance	NS	NS	NS	NS	NS	*
Watering regime (W)						
1/3 FC	0.12	0.13	0.15	3.5	4.2	4.0
2/3 FC	0.14	0.21	0.27	3.8	3.9	5.0
FC	0.16	0.29	0.42	3.9	4.9	7.0
SEM	0.008	0.011	0.018	0.28	0.46	0.82
Significance	*	**	**	NS	NS	*
Significant interactions	Nil	Nil	NxI	Nil	Nil	Nil
CV %	27.8	25.7	30.7	36,4	51.7	75.2

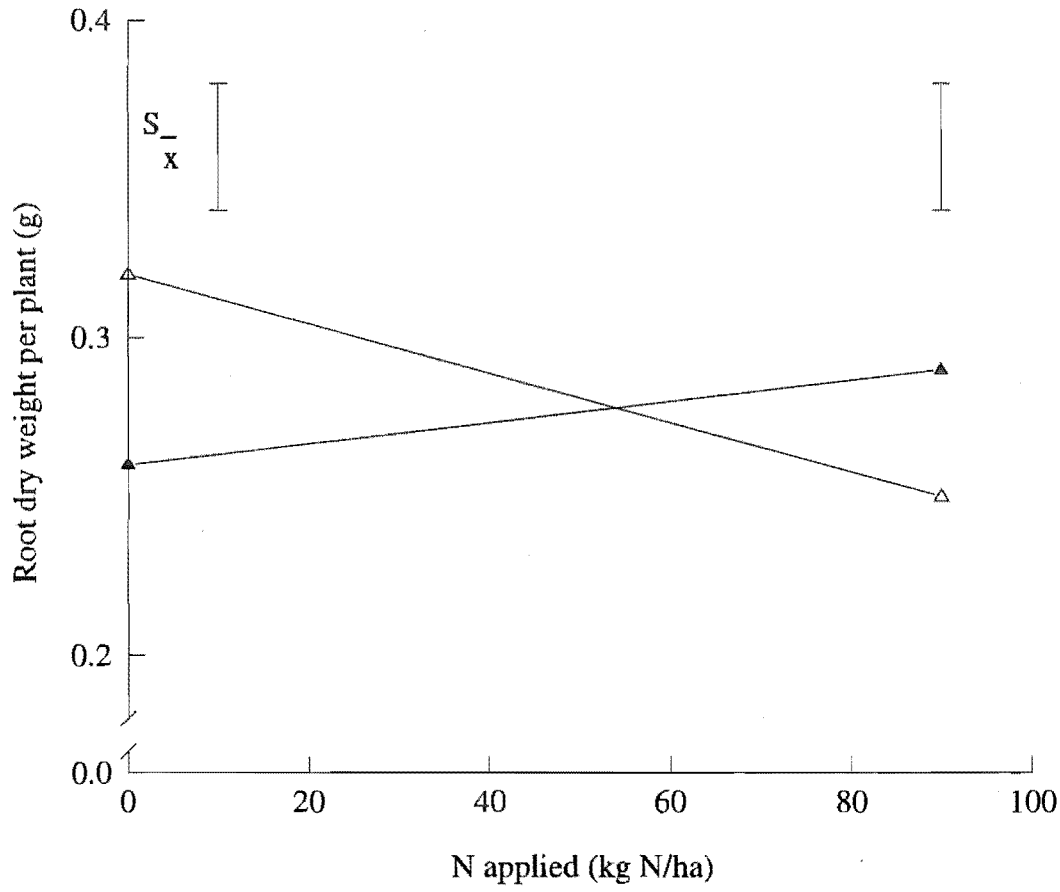

Figure 8: Inoculation by nitrogen interaction on the root dry weight per plant of glasshouse grown chickpeas at 60 DAS.

No inoculation (△), recommended rate (▲).

5.2.2.4 Leaf number per plant

Mean leaf number/plant increased from 24.3 to 27.5 to 37.2 at 30, 45 and 60 DAS respectively. It was significantly ($p<0.05$) increased by additional N and supplemental watering at all harvest dates. Compared to the plants receiving no additional N, the increase with applied N was from 22.4 to 26.1 leaves/plant at 30 DAS, from 22.2 to 32.8 leaves/plant at 45 DAS and from 31.4 to 42.9 leaves/plant at 60 DAS (Table 5.4). Between 30 and 60 DAS, the fully watered plants had between 18 and 74% more leaves/plant than those maintained at 1/3 FC (Table 5.4).

Table 5.4. The effect of nitrogen application, watering regime and *Rhizobium* inoculation on the number of leaves per plant and green area per plant of chickpeas grown in the glasshouse at 30, 45 and 60 days after sowing.

Treatment	Number of leaves per plant			Green area per plant (cm^2)		
Nitrogen (N) kg N/ha	30	45	60	30	45	60
0	22.4	22.2	31.4	75.9	72.3	66.8
90	26.1	32.8	42.9	79.0	93.3	110.7
SEM	1.02	1.54	2.57	3.12	3.27	5.55
Significance	*	**	*	NS	**	**
Inoculation rate (I)						
No inoculation	23.9	27.0	35.1	75.5	77.2	78.0
Recommended	24.6	27.9	39.2	79.4	88.5	99.5
SEM	1.02	1.54	2.57	3.12	3.27	5.55
Significance	NS	NS	NS	NS	*	*
Watering regime (W)						
1/3 FC	23.1	22.7	27.2	62.8	53.1	41.3
2/3 FC	22.4	26.8	37.0	74.5	84.0	90.4
FC	27.3	32.9	47.3	95.2	111.4	134.6
SEM	1.25	1.89	3.15	3.82	4.01	6.79
Significance	*	**	**	**	**	**
Significant interactions	Nil	Nil	Nil	Nil	IxW	NxW
CV %	25.2	33.7	41.5	24.1	23.7	37.5

5.2.2.5 Green area per plant

All three factors significantly ($p<0.05$) influenced green area per plant. Additional N increased green area/plant by 29 and 66% at 45 and 60 DAS respectively (Table 5.4). While the green area of the unfertilized plants declined throughout the duration of growth it increased continuously in plants receiving 90 kg N/ha (Table 5.4). Between 30 and 60 DAS therefore, green area in the fertilized plants increased by 40% from 79.0 cm^2 to 110.7 cm^2 but declined by 12% from 75.9 cm^2 to 66.8 cm^2 in the unfertilized plants. At the first harvest date (30 DAS), green areas of the plants maintained at 1/3 FC and 2/3 FC were 34 and 22% less respectively than those of the well watered plants (Table 5.4). As growth progressed, the difference in green area/plant between the watering regimes widened such that by 60 DAS, green area/plant was more than three times greater in the fully watered plants than in the plants maintained at 1/3 FC. Between 30 and 60 DAS green area/plant declined by 34% in the plants maintained at 1/3 FC but increased by 21 and 41% in the plants maintained at 2/3 FC and FC in that order (Table 5.4).

Inoculation significantly ($p<0.05$) increased green area/plant by 15 and 28% at 45 and 60 DAS respectively (Table 5.4). At 45 DAS there was a significant ($p<0.05$) inoculation by watering regime interaction on green area where the green area of the inoculated plants increased by 53% over the uninoculated plants (Figure 9). The influence of increased N application on green area/plant also depended on the availability of sufficient water at 60 DAS. At field capacity, application of 90 kg N/ha increased green area by 93% from 92.0 cm^2 to 177.2 cm^2 but only by 36% from 76.6 cm^2 to 104.2 cm^2 and 59% from 36.9 cm^2 to 50.7 cm^2 at 2/3 FC and 1/3 FC respectively (Figure 10).

5.2.2.6 Shoot dry weight per plant

At 30 DAS the fully watered plants had shoot dry weights that were 31 and 20% higher than those of the plants maintained at 1/3 FC and 2/3 FC respectively (Table 5.5). While shoot dry weight increased at all watering regimes between 30 and 60 DAS, the increase was greater in the plants maintained at FC which at 45 DAS had dry weights that were 80 and 26% higher than that of the plants maintained at 1/3 FC and 2/3 FC respectively. At 60 DAS, shoot dry

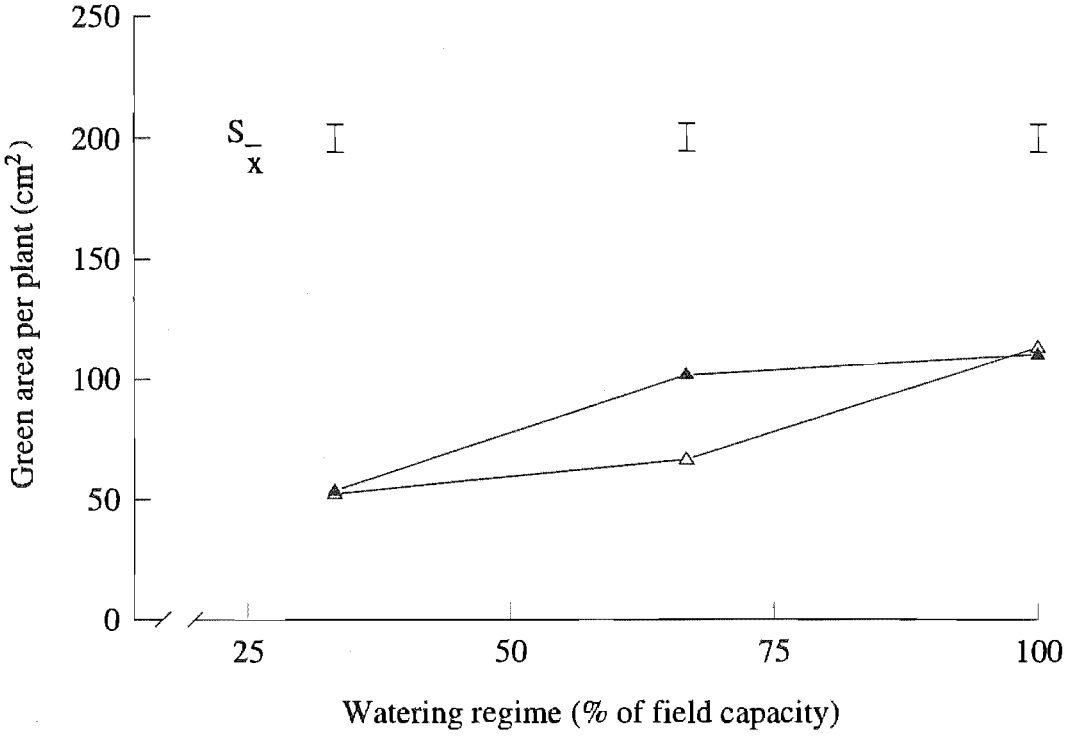

Figure 9: Inoculation by watering regime interaction on the green area per plant of glasshouse grown chickpeas at 45 DAS.

No inoculation (△), recommended rate (▲).

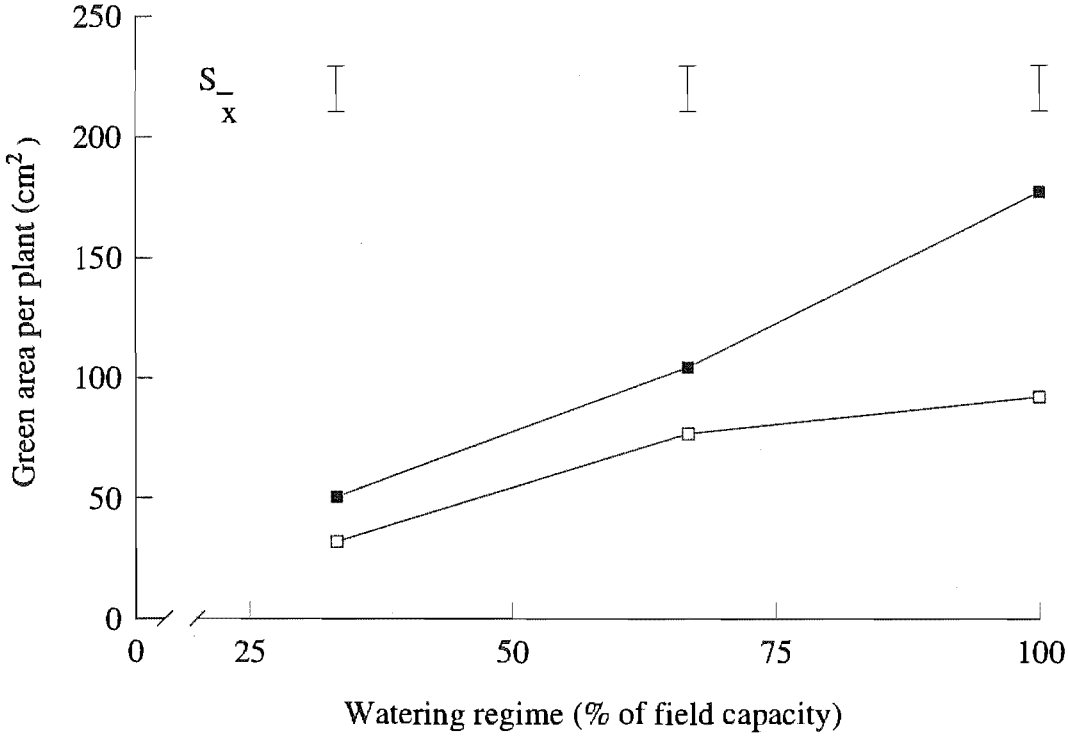

Figure 10: Nitrogen (kg N/ha) by watering regime interaction on the green area per plant of glasshouse grown chickpeas at 60 DAS.

0 N (□), 90 N (■).

weights of the plants maintained at 1/3 FC and 2/3 FC were 41 and 74% that of the fully watered plants respectively. Nitrogen application significantly ($p<0.001$) increased shoot dry weight only at 60 DAS when the shoot dry weights of the plants given no additional N was 71% that of the fertilized plants (Table 5.5). The effects of additional N fertilizer on shoot dry weight depended on the availability of sufficient water at 60 DAS. With 0 kg N/ha, the fully watered plants produced twice (1.2 g/plant against 0.57 g/plant) the shoot dry matter of the plants receiving the lowest amount of water. However with 90 kg N/ha the fully irrigated plants produced nearly 2.7 times (1.9 g/plant against 0.7 g/plant) the shoot dry matter of the plants receiving the least irrigation (Figure 11).

Table 5.5. The effect of nitrogen application, *Rhizobium* inoculation and watering regime on the shoot dry weight of chickpeas grown in the glasshouse at 30, 45 and 60 days after sowing (DAS) and shoot N (%) at 60 DAS.

Treatment	Shoot dry weight per plant (g)			Shoot N (%)
Nitrogen (N) kg N/ha	30 DAS	45 DAS	60 DAS	60 DAS
0	0.36	0.75	0.92	2.07
90	0.37	0.77	1.30	2.70
SEM	0.015	0.030	0.041	0.094
Significance	NS	NS	**	**
Inoculation rate (I)				
No inoculation	0.36	0.75	1.06	2.06
Recommended	0.37	0.77	1.16	2.71
SEM	0.015	0.030	0.041	0.094
Significance	NS	NS	NS	**
Watering regime (W)				
1/3 FC	0.32	0.54	0.63	2.42
2/3 FC	0.35	0.77	1.14	2.36
FC	0.42	0.97	1.55	2.37
SEM	0.019	0.037	0.050	0.094
Significance	**	**	**	NS
Significant interactions	Nil	Nil	NxW	NxI
CV %	25.0	23.7	21.9	9.6

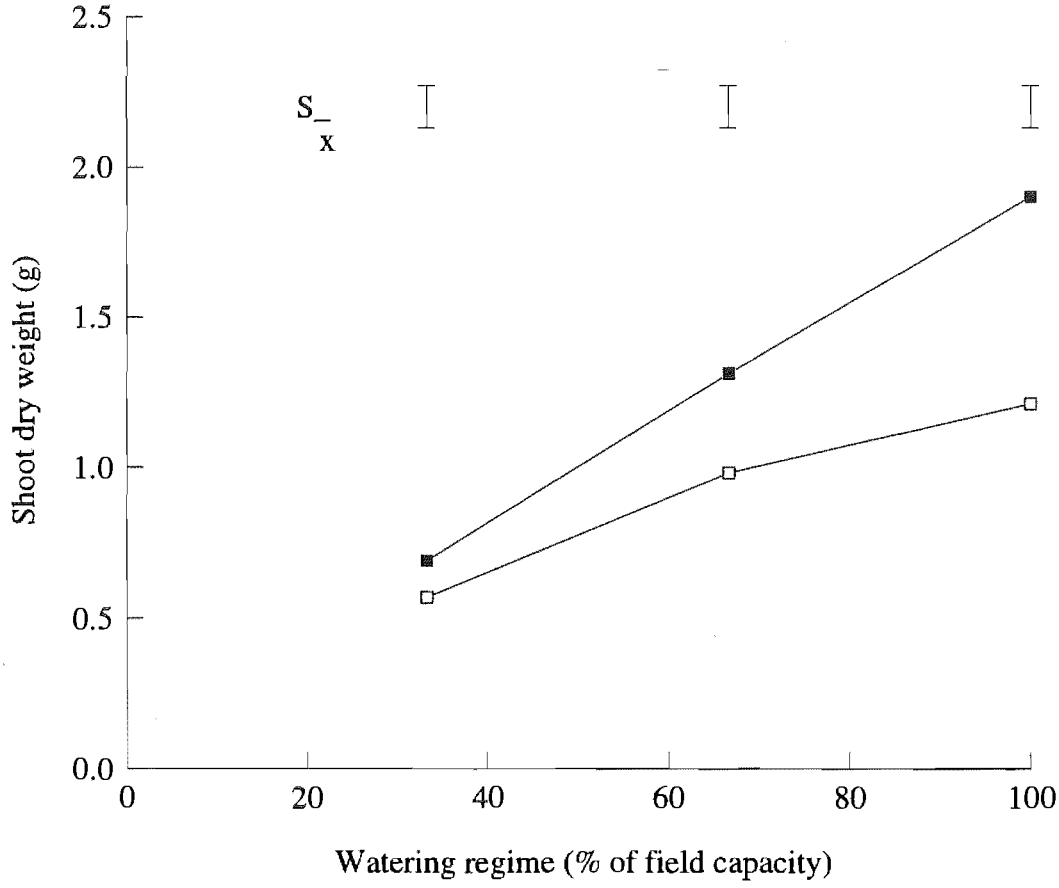

Figure 11: Nitrogen (kg N/ha) by watering regime interaction on the shoot dry weight of glasshouse grown chickpeas at 60 DAS.

0 N (□), 90 N (■).

5.2.2.7 Shoot percent N

The nitrogen content in the shoots at 60 DAS averaged 2.38% and was significantly ($p<0.05$) affected by both nitrogen application and inoculation. Plants provided with additional fertilizer N had 30% more shoot N than those receiving no N, while those inoculated had 32% more shoot N than the uninoculated plants (Table 5.5). The significant ($p<0.05$) nitrogen by inoculation interaction showed that without inoculation, the addition of 90 kg N/ha increased percent shoot N by 82% from 1.46 to 2.65%. However, with additional N, there was no difference in shoot N between the inoculated and uninoculated plants (Figure 12).

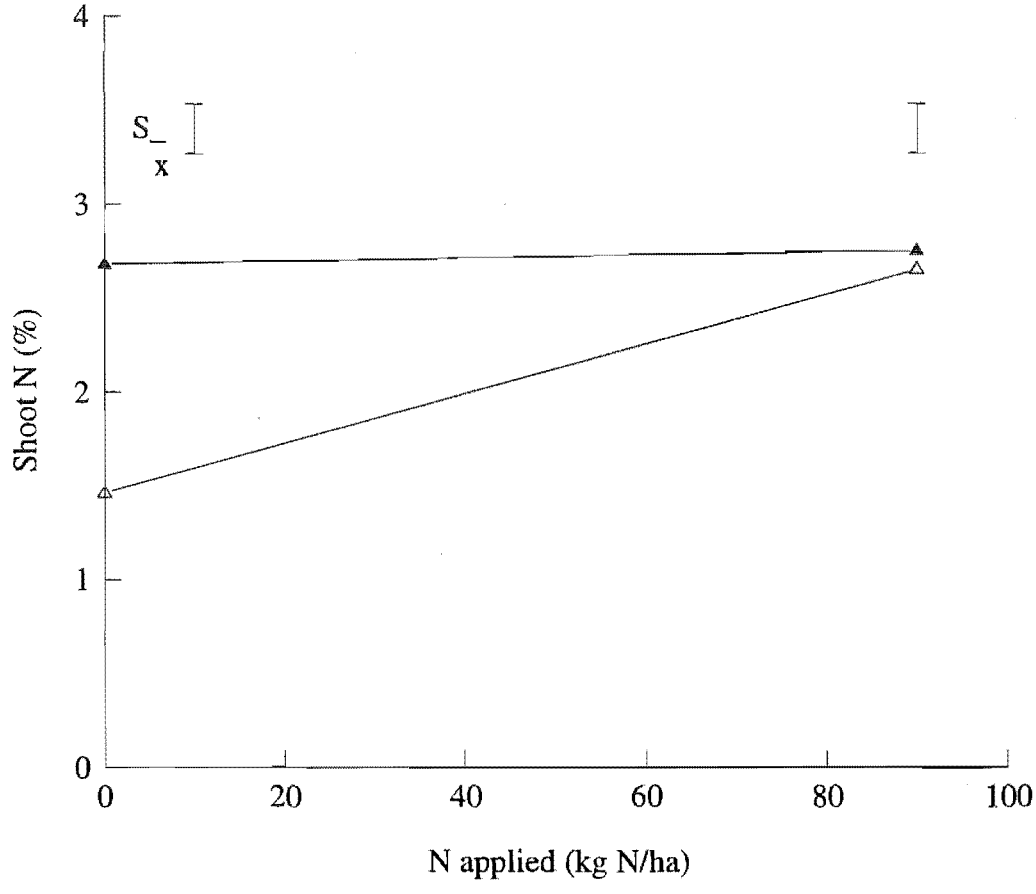

Figure 12: Nitrogen (kg N/ha) by inoculation interaction on shoot N (%) of glasshouse grown chickpeas at 60 DAS.

No inoculation (△), recommended rate (▲).

5.3 Discussion

5.3.1 Nodulation response

5.3.1.1 Inoculation

Where inoculated chickpea has not been sown before, native chickpea rhizobia may be lacking and the objective of legume rhizobial inoculation is to provide sufficient rhizobia for effective nodulation (Hernandez and Hill, 1984; Clark *et al.*, 1988). Although inoculation resulted in a significant nodulation response in both the field (1992/1993 season) and glasshouse experiments, this nodulation was considered inadequate as indicated by the low number of nodules initiated/plant (Tables 5.1 and 5.2). Similar results have been reported in common bean (*Phaseolus vulgaris*) (Graham, 1981; Graham *et al.*, 1982), groundnut (*Arachis hypogaea*) (Nambiar *et al.*, 1982), chickpea and pigeonpea (*Cajanus cajan*) (ICRISAT, 1985).

This contrasts with the prolific nodulation response to inoculation in chickpea obtained by Corbin *et al.* (1977), McNeil *et al.* (1981), Hernandez and Hill (1984) and Zahid *et al.* (1984). Corbin *et al.* (1977) further observed a strong nodulation response to inoculation even at inoculant rates one fifth the recommended. An observation that may have been due to the fresh (a week old) cultures used for inoculation. Both Hernandez and Hill (1984) and Corbin *et al.* (1977) also report using nutrient solutions to provide required macro and micro-nutrients. In the present study nutrient solution was not provided to the growing plants. The response reported by Zahid *et al.* (1984) can be attributed to their alleviation of phosphorus (P) insufficiency by P fertilization and the more favourable growth conditions observed in the winter sowing of chickpeas in the Mediterranean region (Wery *et al.*, 1988).

Several authors (eg. Hamdi and Alla El-Din, 1982) have reported that inadequate nodulation may result from a failure of the rhizobia to establish in the rhizosphere, inappropriate inoculant application method, fungicidal seed treatment or a combination of these factors. Inoculation trials in soybean (*Glycine max* (Merr.)) (Herridge *et al.*, 1984) and groundnut (Nambiar *et al.*, 1982) indicate that the extent of nodulation is related to the size of the rhizobial inoculant in the rhizosphere. No attempt was made to determine the number of rhizobia/g soil following inoculation but there is reason to suspect that this may have been

low. This is because in both growing seasons, chickpea in the field was inoculated with double the recommended rate of inoculum and even then average number of nodules initiated/plant rarely exceeded 10 (Figures 3, 4 and Table 5.1). *Rhizobium* strain CC1192 used in this study although considered a suitable inoculant in Australia (Corbin *et al.*, 1977) and confirmed by Hernandez and Hill (1984) in Canterbury, needs to be evaluated further alongside other strains as recent results suggest that the strain may not be as effective as initially thought (Silsbury, 1989). Furthermore, differences in effectiveness among chickpea rhizobial strains have been reported (Islam, 1981a; Khating and Gonsikar, 1981).

Low soil temperatures during the early part of the 1992/1993 growing season (Table 4.1) compared to the 1993 season (Table 4.2) may have been a major constraint to effective nodulation in the field during that season. On average 1 nodule/plant was recovered on the first harvest date in the 1992/1993 season (Table 5.1) but at a similar period in the following season 8.3 nodules/plant were recovered. In a study involving 30 lines of subterranean clover (*Trifolium subterraneum* L.) inoculated with seven strains of *Rhizobium trifolii*, Brockwell and Robinson (1970) found that nodulation was faster at 22 °C than at 12 °C, a result in agreement with similar studies in common bean (Graham, 1981) and chickpea (Dart *et al.*, 1975). By decreasing root growth and root hair formation, low soil temperature can reduce nodulation as rhizobia gain entry into the roots through the root hairs (Pueppke, 1986). Barrios *et al.* (1963) have reported nodulation failure after a 50% reduction in the length of the primary root of *P. vulgaris* at 12 °C compared with 33 °C.

The conventional seed inoculation method used in these studies may have contributed to the poor nodulation observed. Results from groundnut and chickpea inoculation trials at ICRISAT and ICARDA respectively indicate that excellent nodulation was obtained when *Rhizobium* was added directly to the soil at sowing (Islam and Afandi, 1980; Nambiar *et al.*, 1984). Habish and Ishag (1974), have also reported better nodulation in *P. vulgaris* when soil inoculation rather than seed inoculation was done. Work in Canterbury by Hernandez and Hill (1983) has shown that drilling marble chips containing the desired rhizobial strain can also give good nodulation.

Seed inoculation is still a widely used method of introducing rhizobia into the rhizosphere (Brockwell *et al.*, 1980). However several authors have cast doubts about the effectiveness

of this method when fungicides have to be used with seed at sowing (Rupela and Saxena, 1987). Although there is a dearth of information about the effects of Apron 70 SD used in this study on chickpea nodulation, other fungicides can sometimes reduce both rhizobial populations and nodulation in chickpea (Sprent *et al.*, 1988). The fungicides Thiram (tetramethylthiurum disulphate), PMA (phenylmercuric acetate), PCNB (pentachloronitro-benzene) and Carbendazim (2-(methoxy-carbomyl)-benzomidazole) used at a rate of between 2.5 and 3 g/kg seed adversely affected nodulation in chickpea (Bhattacharyya and Sengupta, 1984). Contrary to these findings, Thomas and Vyas (1984), after evaluating 7 fungicides including Thiram concluded that only Captan significantly reduced the number of nodules. The lower dose (1.5-2.5 g/kg seed) of fungicide used in that study may have influenced the results. If fungicides have to be used on seed, it may be appropriate to incorporate the inoculant directly into the soil instead of applying it onto the seed (Corbin *et al.*, 1977; Nambiar *et al.*, 1984).

A few nodules were recovered from some plants in the uninoculated plots (Table 5.1). This observation has been reported by several authors and the number of nodules/plant have ranged from a few to many (Diatloff, 1970; Khating and Ghonsikar, 1981). While such a response may be attributed to the presence of native rhizobial strains (Graham *et al.*, 1982), it has also been observed in soils free of indigenous rhizobia (McNeil *et al.*, 1981). Rhizobia can be transferred from inoculated plots to the uninoculated by wind (Rupela and Dart, 1980) or water movement (Roughley and Worrall, 1984). Because the experimental site had not been sown to chickpea before, it is plausible to assume that native chickpea rhizobia were lacking and water movement was responsible for the dispersal of the rhizobia.

The significant inoculation by sowing date interaction on nodule number (Figure 2) indicates that the movement of rhizobia from the inoculated to the uninoculated plots occurred to a greater extent in the July than in the September sowing. Rainfall in August of that season (Table 4.1) was more than 3 times the long term mean and it is likely that the soil was at or near field capacity for a considerable length of time hence facilitating the transfer. This is consistent with the observation that chickpea and soybean rhizobia move in saturated soils and at times rhizobial motility may be relatively unimportant compared to their passive movement in flowing water (Breitenbeck *et al.*, 1988; Issa *et al.*, 1993a,b).

5.3.1.2 Nitrogen

With the discovery more than a century ago that peas fix nitrogen, interest in biological nitrogen fixation research (BNF) has widened considerably (Nutman, 1987). One aspect of BNF that has been studied extensively, as exemplified by the numerous reviews (eg. Becana and Sprent, 1987; Streeter, 1988) and research publications (eg. Pate and Dart, 1961; Chen and Phillips, 1977; McNeil, 1982; Streeter, 1985a,b; Silsbury, 1989), is the effect of combined nitrogen (N) on legume nodulation and nitrogen fixation. Results obtained so far indicate no effect (Rys and Phung, 1984), inhibitory (Eaglesham, 1983; Eardly, 1985; Imsande, 1986), or stimulatory (Pate and Dart, 1961; Das, 1982), effects depending on the source of combined nitrogen (Dart and Wildron, 1970), rate of application (Rawsthorne *et al.*, 1985b; Becana and Sprent, 1987), time of application (McNeil, 1982), species (Harper and Gibson, 1984; Jessop *et al.*, 1984) and cultivar (Gibson and Harper, 1985).

Data in Table 5.1 and Table 5.2 show that additional N significantly reduced nodule number per plant albeit only during the early part of the growing season in the field (Table 5.1). These findings confirm earlier observations on the effect of additional N on chickpea nodulation (Hernandez and Hill, 1984; Jessop *et al.*, 1984). However, unlike them, nodule number in the pot experiment was significantly reduced by N application throughout the growth duration (Table 5.2). This could be because Hernandez and Hill (1984) used lower rates of nitrogen (maximum of 30 kg N/ha) while Jessop *et al.* (1984) allowed flushing of solution through the pots to occur. A recovery from the detrimental effects of additional N as observed in nodule number in the field during the 1992/1993 growing season (Table 5.1) has been reported in purple vetch (*Vicia atropurpurea*) (Pate and Dart, 1961) and white clover (*Trifolium repens*) (Rys and Phung, 1984) and is attributed to a reduction in the effective concentration of nitrogen as a result of plant uptake (Dart and Waldron, 1970). As the growing season was wet in the 1992/93 season, leaching of N could also have occurred.

Establishment of functional nodules involves the processes of infection, nodule initiation and development, all of which can be influenced by additional N. It is therefore difficult to determine with certainty the stage(s) that is(are) most susceptible to the detrimental effects of additional N. Available evidence indicate an effect on infection (Streeter, 1988), nodule initiation and development (Sawhney *et al.*, 1985) or both (Gibson, 1987). It is as yet unclear

whether the effects of additional N are due to the external presence of nitrate or its uptake and metabolism (Gibson and Harper, 1985). The data in Table 5.1 and Table 5.2, although inconclusive in themselves, would seem to suggest an external effect because in both cases nodule number increased as the growth duration progressed. If the reverse were the case then a internal effect would be more likely. Harper and Cooper's (1971) work in *Glycine max* (L.) Merr. is also in favour of an external effect. An inhibitory effect of nitrate on early symbiotic events, possibly the *Rhizobium*-legume recognition mechanism has been suggested (Truchet and Dazzo, 1982). In *Trifolium repens*, inoculated with *Rhizobium trifolii*, the concentration and activity of the plant lectin trifoliin has been shown to decline as the concentration of nitrate and ammonium in the rooting medium is increased (Dazzo and Brill, 1978; Sherwood *et al.*, 1984).

5.3.1.3 Water stress - glasshouse experiment

As a rainfed crop in its more traditional growing areas, chickpea experiences increasing moisture stress during its growth (Rupela and Kumar Rao, 1987). Yet little published information exist on the effects of water stress on its nodulation and nitrogen fixation. Most of the available data on the effects of water stress on legume nodulation and nitrogen fixation is derived from the extensive work done on soybean and field bean (Sprent, 1971; 1972a,b; Parkhust and Sprent, 1975; Sprent, 1976; Gallacher and Sprent, 1978). These authors indicate that the availability of water affects the survival, multiplication and movement of rhizobia in the soil (Sprent, 1976) and water stress has been observed to decrease the populations of *R. japonicum* (Al-Rashidi *et al.*, 1982) and *R. leguminosarum* (Pena-Cabriales and Alexander, 1979).

Data in Table 5.2 shows that water supply had no significant effect on nodule number up to 45 DAS. Because plants in all three watering treatments initiated an equal number of nodules at 30 DAS (Table 5.2), it would seem that rhizobial populations were least affected. This agrees with data obtained in *Trifolium subterraneum* where water stress completely inhibited nodulation while having no effect on the number of rhizobia in the rhizosphere (Worrall and Roughley, 1976). That plants in the three watering regimes initiated an equal number of nodules at 30 DAS may have been a result of the full irrigation provided at sowing and the

subsequent additional 50 ml/pot seven days later. In groundnuts, the multiplication of rhizobia and the infection of plant roots has been reported to occur during the short periods of moisture availability following irrigation (Shimshi *et al.*, 1967). For the plants maintained at 1/3 FC, nodule number/plant declined continuously during their growth (Table 5.2). This suggests an effect of water stress on nodule initiation and development. Because water stress retards both root and root hair development (Rupela and Kumar Rao, 1987), it is possible that fewer nodules were initiated and developed on lateral roots as the effects of water stress on nodulation are mediated through a lack of water for cell expansion resulting in nodule shedding (Sprent, 1971; Sprent *et al.*, 1988). Restriction of oxygen diffusion into the nodules may also occur since the effects of moderate water stress can be alleviated by an increase in the oxygen concentration available to the nodules (Pankhurst and Sprent, 1975; Sprent, 1976).

5.3.1.4 Sowing date

In both growing seasons, sowing date had no effect on nodule number per plant, an observation that has been reported in chickpea studies in Australia (Corbin *et al.*, 1977). However, there was variability in nodule number per plant between the two growing seasons. During the 1993 season, more nodules were initiated per plant (Figure 4) possibly due to the more favourable temperatures (Table 4.2). In *Vicia faba*, Andrews (1986b) reported nodulation failure at 10 °C. It would seem therefore that the early part of the 1992/93 growing season was generally unfavourable for nodulation because until November mean daily soil temperatures were less than 10 °C (Table 4.1) hence the lack of variation between the two sowing dates. During the 1993 season the winter was extremely dry and the early spring (September) excessively wet (Table 4.2). Both of these conditions might have masked any differences in nodule number per plant due to sowing date. Nodulation trials in the Mediterranean region however indicate a better response in winter possibly due to the more favourable soil moisture regime (Islam and Afandi, 1980; Islam 1981a).

5.3.2 Dry matter accumulation

5.3.2.1 Mobilization of seed reserves

Material stored in the seed is normally hydrolysed and utilized for growth during seed germination. Because water is essential for the hydrolysis and the subsequent transport of the soluble product (Slatyer, 1973), cotyledon dry weight may be inversely related to the amount of water available in the soil during germination. This was shown to be the case in the present study because at 30 and 45 DAS, the cotyledon dry weights of the fully watered plants were significantly lower than that of the plants maintained at 1/3 FC or 2/3 FC (Table 5.2). This is a clear indication that the fully watered plants mobilized their seed reserves to a greater extent than the plants in the other watering regimes and confirms the important role played by water during seed germination.

Additional N also influenced the mobilization of seed reserves early in the growing period (30 DAS). At this time the cotyledon dry weights of the plants given 90 kg N/ha weighed 31% less than those receiving no added N (Table 5.2). This rapid utilization of seed reserves during early growth by plants provided with additional N has been reported in barley (*Hordeum vulgare* L.) by Metiver and Dale (1977) and is a result of the greater leaf expansion caused by additional N requiring the synthesis of substantial quantities of cell wall and cytoplasm (Hay and Walker, 1989). Because additional N and supplemental watering separately enhanced mobilization of seed reserves (Table 5.2), their interaction could be expected. In this interaction, the greatest mobilization of seed reserves occurred when the plants were maintained at 2/3 FC and given 90 kg N/ha (Figure 7). This result would seem to indicate an overriding influence of water on seed reserve mobilization. Further support for this is provided by the apparent similarity in the amounts of seed reserves mobilized by the plants maintained at field capacity (Figure 7).

5.3.2.2 Root growth

Many studies on root growth indicate that the provision of additional N increases root dry weight (Das, 1982; Jessop *et al.*, 1984; Squire, 1990). Contrary to these results, the main

effect data in Table 5.3 shows that at 30 and 45 DAS, 90 kg N/ha reduced root dry weight/plant by 19 and 17% respectively. Similar findings have been reported in chickpea supplied with 20 mM nitrate (Sawhney *et al.*, 1985). This observation has been attributed to the greater investment of dry matter into both leaves and branches to the detriment of the root with increasing N application (Rawsthorne *et al.*, 1985a). Furthermore, since chickpea reduces and assimilates its nitrogen in the roots, the additional respiratory burden associated with this process could represent a substantial drain on assimilate allocated to the root to the extent that root growth is compromised (Sprent and Thomas, 1984).

The provision of fertilizer N to inoculated chickpeas as indicated in the interaction in Figure 8 increased root dry weight by 12%. Rawsthorne *et al.* (1985a) have found a greater partitioning of assimilate into the roots of chickpea when the plants were dependent on N fixed symbiotically. Similar studies in red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) involving the export of $^{14}CO_2$ have also shown that a greater proportion of the ^{14}C is exported to the roots in inoculated plants compared to those dependent on nitrate (Ryle *et al.*, 1981).

It is possible therefore that irrespective of whether additional N is provided or is not provided, more assimilate would normally be allocated to the roots of inoculated plants in readiness for nodule development and maintenance as well as the transport of fixation products. However, when additional N is not available the source size is limited and most assimilate to the roots is utilized in the development and maintenance of nodules, hence reduced root dry weight as found in the present study. This concurs with reports in cowpea which indicate that the presence of nodules reduces early root growth (Summerfield *et al.*, 1977). When additional N is provided the source size is enhanced but nodulation is inhibited (Streeter, 1985a,b), hence assimilate allocated for nodule development and maintenance is diverted into root growth. Supplemental watering increased root dry weight throughout the growth period (Table 5.3) and confirms similar findings in cowpea (Turk and Hall, 1980b). These increases in root dry weight signify more photosynthate allocation into the roots as a result of greater source size.

5.3.2.3 Leaf growth

Since the availability of nitrogen is the factor that most limits shoot growth when other factors are optimum (Andrews, 1986a), its effects on leaf growth have been studied extensively in both legumes (eg. Jessop *et al.*, 1984; McKenzie *et al.*, 1992) and cereals (eg. Hay and Walker, 1989; Andrews *et al.*, 1991). There is general agreement that supplemental N increases leaf area (Metiver and Dale, 1977; Summerfield *et al.*, 1977). The data in Table 5.4 shows that additional N increased green area per plant at 45 and 60 DAS by 29 and 66% respectively, an increase that is within the range reported for a similar crop by McKenzie *et al.* (1992). At the cellular level, additional N enhances cell division and enlargement (Hay and Walker, 1989), increases photosynthetic pigments and hence leaf photosynthesis (Squire, 1990). There is also a disproportionate allocation of dry matter in favour of the leaves with increasing N application (Rawsthorne *et al.*, 1985a).

Hay and Walker (1989) have concluded that the increase in green area in plants provided with additional N results from increased branching. Data on branch number confirms this observation. At 30 and 60 DAS, the increase in branch number with additional N was 21 and 68% respectively while the corresponding increase in leaf area was 29 and 66% (Table 5.3 and 5.4). Green area/plant in the plants receiving 90 kg N/ha increased by 40% between 30 and 60 DAS. However, over the same period, leaf number increased by 64% (Table 5.4), indicating ontogenetic decline in leaf size along the canopy (Squire, 1990). Data in Table 5.4 suggest that N deficiency affected leaf area more than leaf number. This is because while leaf number increased by 40% in the unfertilized plants between 30 and 60 DAS, leaf area declined by 12%. The decline in green area/plant was therefore due to the initiation of fewer leaves, but more importantly these leaves were smaller in size. Similar results have been obtained in lentils (Turay, 1993). In that particular study, in addition to reducing leaf number and total leaf area per plant, N deficiency also delayed leaf appearance.

The effects of water stress on leaf growth are well documented (eg. Hsiao and Acevedo, 1974; Begg and Turner, 1976; Turk and Hall, 1980a,b; Eck *et al.*, 1987). In the present study, green area/plant was always higher in the fully watered plants (Table 5.4). Similar results have been reported in cowpea (Turk and Hall, 1980a) and chickpea (Saxena *et al.*, 1983). Water stress effects on green area are manifested initially at the cellular level because up to

90% of the fresh weight in most plant organs is water with 60 to 90% of this amount found within the cells and providing turgidity (Turner and Burch, 1983). Because the influx of water into the cell is responsible for the initial cell expansion, reductions in cell size are normally correlated with reductions in water potential of the growth medium (Simpson, 1981; Hay and Walker, 1989). A reduction in cell size due to water stress reduces the rate at which leaves expand and consequently leaf area (Squire, 1990). This reduces photosynthesis as a result of interruption in carbon dioxide supply by stomatal closure and less photosynthetically active radiation being intercepted (Eck *et al.*, 1987). In this study sensitivity to water stress increased as green area/plant increased (Table 5.4). A similar response has been reported in *Glycine max* (L.) Merr. cv Braxton (Hoogenboom *et al.*, 1987) and may be due to the increased transpirational demand as the canopy size expands.

It may not be physiologically correct to compare the effects of N deficiency and those of water stress but it is worth noting that between 30 and 60 DAS, 0 N reduced green area/plant by 12% while over the same period 1/3 FC reduced it by 34% (Table 5.4). This suggests that water stress may have a greater influence on green area/plant. The data on the watering regime by nitrogen interaction (Figure 10) provides further support for this assertion. The effects of water stress on green area can be linked to fewer branches and leaves/plant (Table 5.3 and 5.4) as has been suggested by Saxena *et al.* (1983). Summerfield *et al.* (1977) found that inoculated cowpea plants were larger and more leafy than the corresponding uninoculated plants. A similar observation was made at 45 and 60 DAS when inoculation increased green area/plant by 15 and 28% respectively (Table 5.4). This was due to the availability of fixed N which enhanced branching (Table 5.3).

5.3.2.4 Shoot growth

Because the effects of water stress on leaves are a reduction in number, rate of expansion and total leaf area (Simpson, 1981), water stressed plants have fewer and smaller leaves (Farah, 1981; Table 5.4). Inevitably therefore, water stress will result in a reduction in shoot growth and hence shoot dry weight as shown by the data in Table 5.5. It has been suggested that plant growth is controlled directly by plant water deficits and only indirectly by soil water deficits (Begg and Turner, 1976). The proportion of water that passes through the canopy

therefore is related to the amount of dry matter accumulated and any reduction in transpirational water use results in a reduction in the dry matter accumulated (Turner and Burch, 1983; Hsiao, 1990). Since less water was lost through transpiration by the water stressed plants, consequently less dry matter was accumulated (Table 5.5). Inoculation failed to increase shoot dry weight contrary to reports from other chickpea experiments (Jessop *et al.*, 1984; Hernandez, 1986). This could have been due to the higher amount of N applied in the present study unlike the lower level of 30 kg N/ha provided by Hernandez (1986).

5.3.2.5 Shoot percent nitrogen

Experimental data indicate a nitrogen content of about 5% in chickpea shoots prior to the period of rapid N mobilization (Saxena and Sheldrake, 1980; Khanna-Chopra and Sinha, 1987). Because growth was terminated at 60 DAS, it is unlikely that N accumulation had peaked by then hence the low average N content of 2.38%. There was a striking similarity in the shoot percent N of the plants receiving 90 kg N/ha and those inoculated with the recommended rate of *Rhizobium* inoculant (Table 5.5). However, more interesting are the results of the significant nitrogen by inoculation interaction which suggest that on the basis of shoot percent N, inoculated plants fixed an amount of nitrogen equivalent to that provided by the assimilation of 90 kg N/ha (Figure 12).

5.4 Conclusions

The findings from this study have clearly shown that under both field and glasshouse conditions, additional nitrogen will reduce the number of nodules initiated by chickpea. However, the poor nodulation response to rhizobial inoculation suggests that more strains should be evaluated to ascertain those best suited to the Canterbury environment before an optimum level of *Rhizobium* inoculant application is determined. Mobilization of seed reserves was also shown to depend on additional nitrogen and water availability, indicating that this may be one of the mechanisms by which these treatment factors enhance early growth in chickpea. Water stress reduced the number of nodules initiated per plant and affected green area more than leaf number.

CHAPTER 6

RESULTS AND DISCUSSION - GROWTH, DEVELOPMENT AND YIELD

6.1 Seasonal dry matter accumulation

Dry matter (DM) accumulation in both sowing dates followed a sigmoid curve (Figure 13). Maximum dry matter yield averaged 10.35 t/ha and was significantly ($p<0.05$) affected by sowing date at all harvest dates. The July-sown crop produced 11.2 t/ha, while the September sown crop produced a maximum of only 9.5 t/ha (Table 6.1). The time to maximum dry matter accumulation varied between the two sowing dates. While the July-sown plants took 214 days to attain maximum dry matter the September sowing took only 125 days.

6.2 Total dry matter accumulation

At final harvest, total dry matter (TDM) accumulated averaged about 7 t/ha. It was not significantly influenced by any factor but the plants given 90 kg N/ha accumulated 7.4 t/ha which was 12% more TDM than those receiving no additional N (Table 6.1).

6.3 Seed yield, harvest index and yield components

5.3.1 Seed yield

At final harvest, seed yield averaged 2.87 t/ha. The July sowing yielded less (2.7 t/ha) than the September-sown crop (3.0 t/ha) but the difference was again not significant (Table 6.1). Seed yield was also not significantly influenced by either inoculation or nitrogen application.

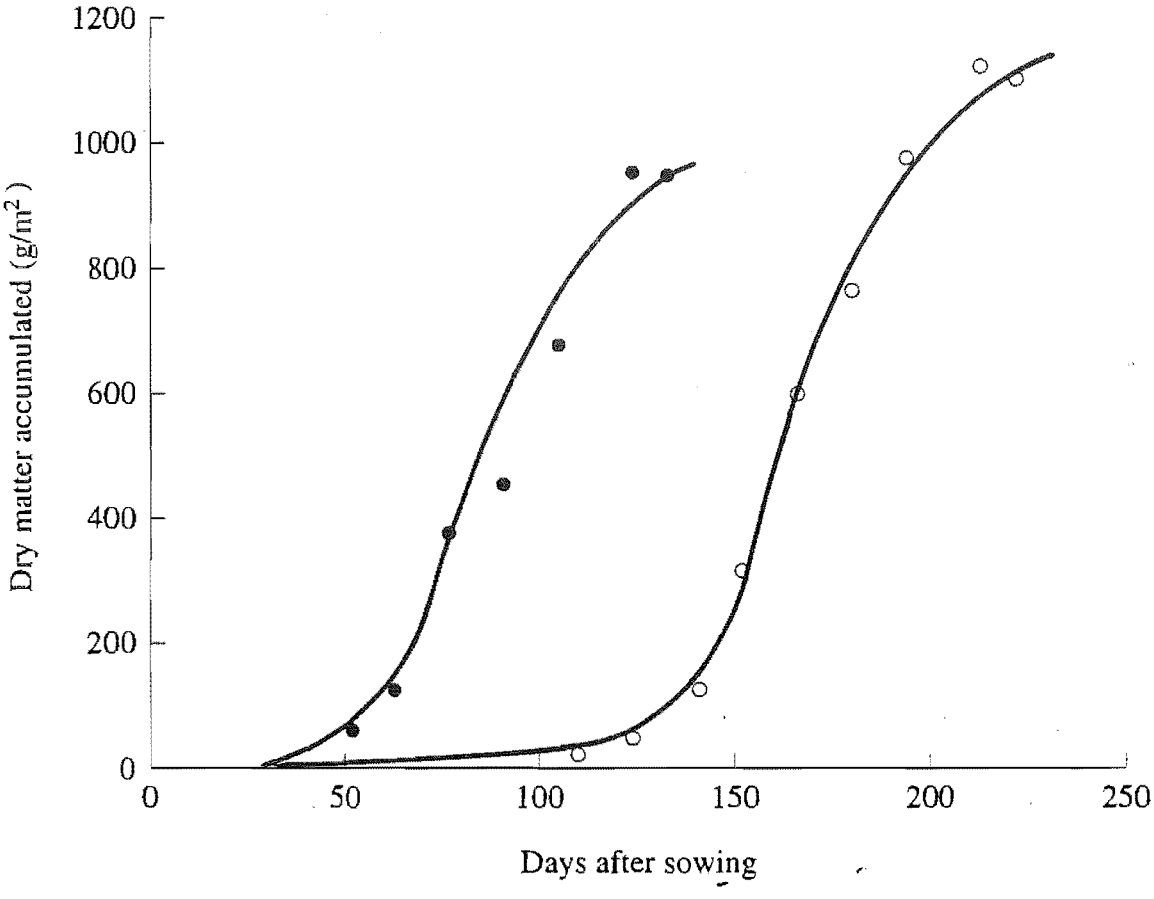

Figure 13: The effect of sowing date on the dry matter accumulated by chickpeas sown on two dates in Canterbury, 1992/93.

July (○), September (●).

Table 6.1. The effect of nitrogen application and *Rhizobium* inoculation on maximum dry matter (DM), total dry matter (TDM), seed yield and harvest index (HI) of chickpeas sown on two dates in Canterbury during the 1992/93 season.

Treatment	Maximum DM (t/ha)	TDM (t/ha)	Seed yield (t/ha)	HI
Nitrogen (N) kg N/ha				
0	10.2	6.6	2.8	0.29
15	9.6	7.1	2.9	0.31
45	10.5	7.0	2.7	0.28
90	11.1	7.4	3.0	0.28
SEM	0.50	0.25	0.11	0.017
Significance	NS	NS	NS	NS
Inoculation rate (I)				
No inoculation	11.0	6.7	2.7	0.26
Recommended	10.3	7.2	2.9	0.30
Double recommended	9.7	7.3	3.0	0.31
SEM	0.44	0.22	0.09	0.015
Significance	NS	NS	NS	*
Sowing date (S)				
July	11.2	6.8	2.7	0.25
September	9.5	7.3	3.0	0.33
SEM	0.36	0.34	0.15	0.012
Significance	**	NS	NS	**
Significant interactions	Nil	Nil	Nil	Nil
CV %	20.7	15.1	15.7	24.6

6.3.2 Harvest index

A mean harvest index of 0.29 was attained in both sowing dates. It was significantly ($p<0.05$) increased by *Rhizobium* inoculation from 0.26 to 0.30 to 0.31 at no inoculation, inoculation with the recommended rate and inoculation with double the recommended rate respectively (Table 6.1). Harvest index also increased as sowing date was delayed from July (0.25) to September (0.33).

6.3.3 Yield components

6.3.3.1 Pods per plant

A mean population of 56 plants/m^2 was achieved. All three factors significantly ($p<0.05$) affected the number of pods/plant. The July sowing produced 37.2 pods/plant which was 29% more than the September sowing while plants inoculated with double the recommended rate of inoculum yielded 18% more pods/plant than the uninoculated plants (Table 6.2). Additional N (90kg N/ha) also increased pod yield from 30.8 to 36.7 pods/plant, a 19% increase compared to the treatment that received no added N.

6.3.3.2 Seeds per pod and thousand seed weight

On average 158.7 seeds were produced per m^2 from 165.1 pods/m^2, giving 0.96 seeds/pod. The number of seeds/pod increased significantly ($p<0.05$) from 0.93 in the July sowing to 0.99 in the September sowing (Table 6.2). Nitrogen application and *Rhizobium* inoculation did not significantly affect the number of seeds per pod. The thousand seed weight averaged 190 g and was not significantly affected by any of the treatments.

6.4 Growth analysis

The July sowing had a weighted mean absolute growth rate (WMAGR) of 14.0 g/m^2 per day which was 9% higher than that of the September sown crop (Table 6.3). The weighted mean absolute growth rate increased with increasing N application from 11.9 to 12.4 to 13.4 to 13.7 g/m^2 per day, at 0, 15, 45 and 90 kg N/ha. Maximum crop growth rate (C_m) increased as sowing date was delayed from 17.6 g/m^2 per day in the July-sown crop to 19.5 g/m^2 per day in the September sowing. It also increased with increasing N application from 18.2 g/m^2 per day without additional N to 20.6 g/m^2 per day when 90 kg N/ha was provided (Table 6.3). Both additional N and delayed sowing decreased the duration of the period of exponential growth, from 79.4 days in the July sowing to 71.6 days when the crop was sown in September and 75.1 days when no N was provided to 71 days when 90 kg N/ha was given (Table 6.3).

Table 6.2. The effect of nitrogen application and *Rhizobium* inoculation on the number of pods per plant, seeds per pod and thousand seed weight of chickpea sown on two dates in Canterbury during the 1992/93 season.

Treatment	Pods per plant	Seed per pod	1000 seed weight
Nitrogen (N) kg N/ha			
0	30.8	0.94	208.9
15	34.2	0.96	187.4
45	30.4	0.93	177.4
90	36.7	1.00	187.4
SEM	1.78	0.029	8.55
Significance	*	NS	NS
Inoculation rate (I)			
No inoculation	29.8	0.92	184.2
Recommended	34.0	0.96	190.8
Double recommended	35.3	0.99	195.8
SEM	1.54	0.025	7.4
Significance	*	NS	NS
Sowing date (S)			
July	37.2	0.93	185.8
September	28.9	0.99	194.8
SEM	1.26	0.020	6.04
Significance	**	*	NS
Significant interactions	Nil	Nil	Nil
CV %	22.9	12.7	19.1

Table 6.3. The effect of sowing date and nitrogen application on the weighted mean absolute growth rate (WMAGR), maximum growth rate (C_m) and duration of exponential growth (DUR) in chickpeas sown in Canterbury during the 1992/93 season.

Treatment	WMAGR ($g/m^2/d$)	C_m ($g/m^2/d$)	DUR (days)
Sowing date			
July	14.0	17.6	79.4
September	12.9	19.5	71.6
Nitrogen application (kg N/ha)			
0	11.9	18.2	75.1
15	12.4	18.9	66.9
45	13.4	20.1	73.3
90	13.7	20.6	71.0

6.5 Leaf area index

Leaf area increase was very slow during early vegetative growth and it was not until 144 DAS that the July-sown crop attained a leaf area index (LAI) of 1 (Table 6.4). Sowing date significantly ($p<0.001$) affected LAI only on the first measurement date when the July sowing had an LAI almost three times greater than that of the September sowing (Table 6.4). While maximum LAI in the September sowing was achieved after 85 DAS, it was not until 174 DAS that the July sowing attained its maximum LAI. Nitrogen application did not significantly affect LAI early in the growing season. However, from 24/12/92 increasing N application gave increased LAI. At 24/12/92, plants fertilized with 90 kg N/ha had an LAI of 3.70 which was 17, 22 and 36% higher than the LAI of the plants receiving 45, 15 and 0 kg N/ha in that order. Towards the end of the growing season, leaf senescence was delayed by N application. The plants given 90 kg N/ha had an LAI of 2.72 which was 14, 16 and 25% higher that of the plants receiving 15, 45 and 0 kg N/ha respectively (Table 6.4).

Table 6.4. The effect of nitrogen application and *Rhizobium* inoculation on the leaf area index of chickpea sown on two dates in Canterbury during the 1992/93 season.

	Leaf area index on harvest date		
Treatment	24/11/92	24/12/92	26/1/93
Nitrogen (N) kg N/ha			
0	0.66	2.72	2.18
15	0.70	3.04	2.39
45	0.71	3.15	2.34
90	0.72	3.70	2.72
SEM	0.063	0.130	0.097
Significance	NS	**	*
Inoculation rate (I)			
No inoculation	0.71	3.14	2.32
Recommended	0.74	3.22	2.57
Double recommended	0.64	3.10	2.34
SEM	0.055	0.112	0.084
Significance	NS	NS	NS
Sowing date (S)			
July	1.04	3.26	2.32
September	0.35	3.04	2.49
SEM	0.045	0.092	0.069
Significance	**	NS	NS
Significant interactions	Nil	Nil	Nil
CV %	38.5	17.5	17.1

6.6 Photosynthetically active radiation (PAR) intercepted

Throughout the growing season, PAR intercepted was influenced by both N application and sowing date. The amount of PAR intercepted by the canopy increased as leaf area increased and reached a maximum of 9.3 MJ/m^2 per day at the time maximum LAI was recorded.

Nitrogen application significantly ($p<0.001$) increased maximum daily PAR intercepted from 9.0 to 9.2 to 9.4 and 9.6 MJ PAR/m^2 per day in the 0, 15, 45 and 90 kg N/ha plots respectively (Table 6.5).

Table 6.5. The effect of nitrogen application and *Rhizobium* inoculation on maximum and total PAR intercepted and the utilization coefficient (calculated with maximum DM) of chickpeas sown on two dates in Canterbury during the 1992/93 season.

Treatment	Maximum PAR intercepted (MJ/m^2/d)	Total PAR intercepted (MJ PAR/m^2)	Utilization coefficient
Nitrogen (N) kg N/ha			
0	9.0	725	1.53
15	9.2	736	1.43
45	9.4	750	1.52
90	9.6	760	1.59
SEM	0.09	12.4	0.077
Significance	**	* (l)	NS
Inoculation rate (I)			
No inoculation	9.2	736	1.62
Recommended rate	9.4	756	1.49
Double recommended	9.3	736	1.46
SEM	0.08	10.7	0.067
Significance	*	NS	NS
Sowing date (S)			
July	9.4	810	1.48
September	9.2	675	1.56
SEM	0.06	8.8	0.055
Significance	*	**	NS
Significant interactions	Nil	Nil	Nil
CV %	4.0	7.1	21.6

l= significant linear contrast at $p<0.05$

Rhizobium inoculation significantly increased ($p=0.05$) maximum daily PAR intercepted with the plants inoculated with the recommended rate intercepting 9.4 MJ PAR/m^2 per day against the 9.2 MJ PAR/m^2 per day intercepted by the uninoculated plants (Table 6.5). Total PAR intercepted was significantly ($p<0.05$) affected by sowing date. Over the entire growing season an average of 743 MJ PAR/m^2 was intercepted. However, the July sowing intercepting 20% more PAR than the September sowing (Table 6.5). There was a significant ($p<0.05$) linear increase in total PAR intercepted with increasing N application (Table 6.5).

6.6.1 Dry matter accumulation and PAR intercepted

Dry matter accumulation in both sowing dates was closely related to intercepted PAR and the regressions accounted for 98% or higher of the variance (Figure 14). At the time of maximum dry matter production, the utilization coefficient was not significantly affected by any treatment and the overall mean was 1.52 g DM/MJ PAR (Table 6.5). However, over the entire growing season the overall utilization coefficient was very similar for both sowings (Figure 14) with a mean of 1.64 g DM/MJ PAR.

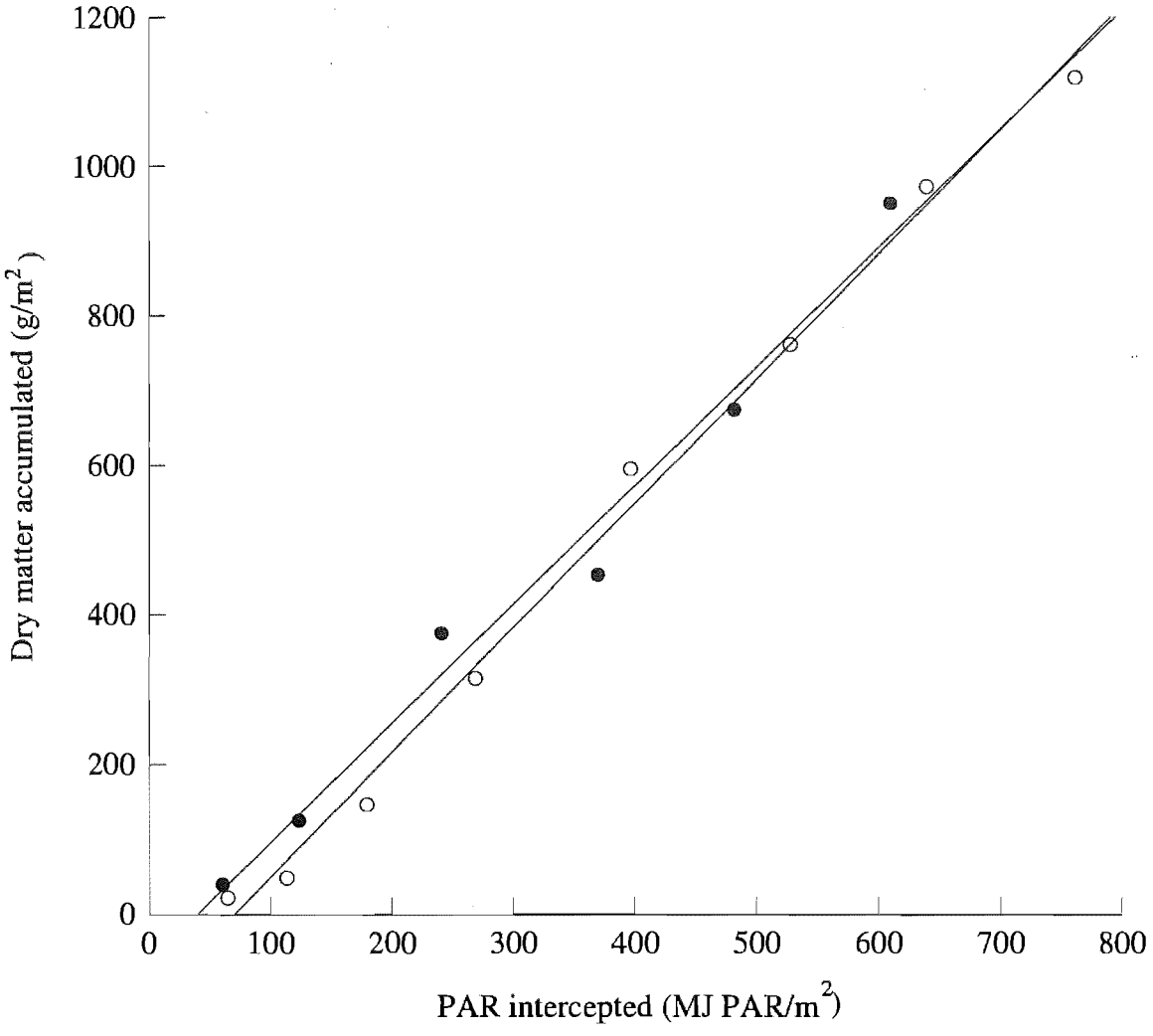

Figure 14: The relationship between dry matter accumulated and PAR intercepted by chickpeas sown in July (○) and September (●) in Canterbury, 1992/93.

(○) $y = 1.67x - 117.7$ ($R^2 = 99.4\%$)

(●) $y = 1.59x - 63.9$ ($R^2 = 98.0\%$)

6.7 Discussion

6.7.1 Dry matter accumulation

As in lentils (McKenzie and Hill, 1990) and faba bean (Herdina and Silsbury, 1990), seasonal dry matter accumulation followed a sigmoid curve at both sowing dates (Figure 13). Because of the low temperatures during winter (Table 4.1), the July-sown crop accumulated dry matter much slower than the September sowing. For example up to 118 DAS, the July sown plants had accumulated only 21.9 g/m^2. Evidence from other chickpea experiments (eg. Khanna-Chopra and Sinha, 1987) confirm this exceptionally slow rate of dry matter accumulation long after sowing which is a result of the very close relationship between leaf area development and dry matter accumulation (Saxena N.P., 1984).

Seasonal dry matter accumulation was strongly influenced by sowing date as found in chickpea (McKenzie *et al.*, 1992) and lentils (McKenzie and Hill, 1990; Turay, 1993) grown under Canterbury conditions. The July sowing accumulated 18% more maximum dry matter than the September sowing (Table 6.1) because it was in the field for longer and intercepted more photosynthetically active radiation (Hughes *et al.*, 1987). The cooler temperatures under which vegetative growth occurred have also been shown to prolong growth since flowers fail to set pods (Saxena *et al.*, 1983). In Canterbury, work on lentils and chickpea indicates that additional nitrogen and rhizobial inoculation can increase maximum dry matter accumulated (Hernandez, 1986; Turay, 1993). In the present study neither additional N or rhizobial inoculation had any effect on maximum dry matter (Table 6.1). This may have been either due to the poor nodulation response to inoculation (Table 5.1) or the mineralization of organic nitrogen with rising spring temperatures (Sprent and Thomas, 1984).

An average final total dry matter accumulation of 7 t/ha is similar to that obtained in lentil and chickpea sown in May in Canterbury (McKenzie 1987; McKenzie *et al.*, 1992). A large proportion of the dry matter accumulated by chickpea is lost in dropped leaves (Saxena and Sheldrake, 1980). Khanna-Chopra and Sinha (1987) estimate a loss in dry matter of between 20% and 30%. The data in Table 6.1 shows that about 32% of the dry matter that had been accumulated by the time maximum dry matter was recorded had been lost at final harvest. This slightly higher value is due to the severe drought that normally occurs in Canterbury

during January and February (McKenzie and Hill, 1990).

6.7.2 Seed yield

The mean seed yield of 2.87 t/ha obtained compares well with the 2.7 t/ha reported by Hernandez and Hill (1985) but is less than the 3.5 t/ha obtained by McKenzie *et al.* (1992). This is a relatively high yield compared with the average of 0.7 t/ha in the traditional chickpea growing areas (Summerfield *et al.*, 1980; Nene, 1987). For the Indian sub-continent the low seed yields are normally due to a failure of many of the seeds to germinate because of inadequate moisture in the seed bed (Saxena and Sheldrake, 1980). Seed yield did not respond to either inoculation or additional nitrogen (Table 6.1). This contrasts with the results obtained in lentils (Turay, 1993) and chickpea (McKenzie and Hill, 1994) in Canterbury. Summerfield *et al.* (1977) and Hernandez (1986) have also obtained seed yield increases after inoculating cowpea and chickpea respectively. While the poor nodulation reported earlier could be a reason for the lack of response in seed yield to inoculation, the failure of additional N to give seed yield increases is an indication that soil N levels were probably high.

Work on lentils in Canterbury has shown that sowing date has the greatest effect on seed yield. Seed yield declined as the sowing date was advanced from autumn to spring (McKenzie and Hill, 1990). Similar studies in Australia indicate that while early sown chickpeas may accumulate more dry matter, this does not normally translate into higher seed yields (Siddique and Sedgley, 1986). In the present study, the July sowing accumulated 18% more maximum total dry matter than the September sowing, however, both sowings ended up with a similar seed yield (Table 6.1). McKenzie *et al.* (1992) have attributed this response to both flower and seed abortion in the early sowing which flowers when night temperatures are still low. Large increases in seed yield are possible in the Mediterranean region when chickpea is sown in winter because growth duration is lengthened and the reproductive period occurs under more favourable environmental conditions (Saxena and Goldsworthy, 1988). Harvest index is therefore improved (Saxena *et al.*, 1990). Winter sowing did not give similar yield increases because harvest index declined with early sowing (Table 6.1). This confirms results from earlier chickpea experiments in Canterbury (McKenzie *et al.*, 1992; McKenzie and Hill, 1994).

6.7.3 Harvest index

Like lentils (McKenzie, 1987), harvest index in chickpea varies widely between and within years (Siddique and Sedgley, 1986). In this study, the mean harvest index of 0.29 obtained is lower than that reported by Hernandez (1986) for a chickpea crop in Canterbury and the range of 0.35 to 0.50 commonly obtained in the traditional chickpea growing areas (Saxena N.P., 1984). While McKenzie *et al.* (1992) obtained a 12% increase in harvest index with application of 100 kg N/ha, the data in Table 6.1 indicates additional N had no effect on harvest index in this experiment. The decline in harvest index with early sowing is consistent with the results of other workers (eg. McKenzie and Hill, 1994). Harvest index in most grain legumes is generally lower than that for cereals because during the period of rapid seed growth, accumulation of dry matter and nitrogen are concurrent (Saxena and Sheldrake, 1980). A high harvest index would therefore mean an even higher nitrogen harvest index (Lawn, 1989).

6.7.4 Yield components

6.7.4.1 Number of pods/plant

Pod yield was significantly ($p<0.001$) influenced by all three treatment factors. Both additional N at 90 kg N/ha and inoculation with double the recommended rate of inoculum increased pods/plant by 19 and 18% respectively (Table 6.2). The increase in pods/plant occurs because of the increased leaf area with additional N being associated with more reproductive nodes (Saxena N.P., 1984). A greater leaf area also results in a corresponding increase in assimilate supply which has been reported to determine pod number in field bean (Husain *et al.*, 1988b). Sowing date had the most profound effect on the number of pods/plant with the July sowing yielding 29% more pods/plant compared with the September sowing (Table 6.2). Keatinge and Cooper (1983) have also obtained a similar result from a winter planted crop in northern Syria. This being due to the winter sown crop making rapid early spring growth and intercepting more solar radiation, therefore producing more pods/unit area (McKenzie and Hill, 1990). Because seed yield between the two sowing dates was similar (Table 6.1), the July sowing must have had more empty pods.

6.7.4.2 Number of seed per pod

Only significantly affected by sowing date, the number of seeds/pod averaged 0.96. The September sowing had 0.99 seeds/pod compared with the 0.93 seeds/pod in the July sowing (Table 6.2). A similar response has been observed in chickpea experiments in south-western Australia and may be due to over investment of dry matter in the stems and leaves by the early sown plants (Siddique and Sedgley, 1986). Being an indeterminate crop, competition between vegetative and reproductive structures for assimilate supply may be sufficiently intense in the early sowing, because of the greater vegetative growth, to the extent that seed abortion occurs (Goldsworthy, 1984).

6.7.5 Growth analysis

6.7.5.1 Weighted mean absolute growth rate (WMAGR)

As the mean growth rate over the period when the crop accumulated most of its total dry matter, the WMAGR in the July sowing of 14.0 g/m^2 per day was 9% higher than that of the September sown crop (Table 6.3). This mean growth rate compares favourably with the 13.6 g/m^2 per day obtained in a crop of lentil sown at a similar time of year in Canterbury (McKenzie, 1987) and is within the range of 11 to 17.1 g/m^2 per day reported by McKenzie and Hill (1990) for autumn/winter sown lentil. Because the July sown crop was already established, by the time temperatures became favourable for growth in October-November, the WMAGR of this crop was higher than that of the September sowing. An increased WMAGR with increased N application, as observed in this study, has also been reported in lentils grown in Canterbury (Turay, 1993).

6.7.5.2 Maximum crop growth rate (C_m)

Monteith (1972, 1978) gives the maximum crop growth rates for C_4 and C_3 crop plants in the temperate region as 22 g/m^2 per day and between 13 and 19 g/m^2 per day respectively. In the present study C_m was 17.6 g/m^2 per day when plants were sown in July and 19.5 g/m^2 per day

when sowing was delayed to September (Table 6.3). This range is well within that given by Monteith (1972, 1978) and is above the maximum crop growth rate of 16 g/m^2 per day in *Vicia faba* L. cv. Maris Bead reported by Husain *et al.* (1988b). Lower values of between 8 and 14 g/m^2 per day have been recorded in chickpea crops at Hyderabad and New Delhi respectively (Saxena, 1984 N.P.; Khanna-Chopra and Sinha, 1987). Differences in environmental conditions such as high night temperatures and or short days, between Canterbury and these sites in India are probably responsible for this observed variation (Pandey, 1980).

6.7.5.3 Duration of the period of exponential growth

From the data in Table 6.3, the duration of the period of exponential growth declined from 79.4 days in the July sowing to 71.6 days in the September sowing. Other studies in Canterbury (eg. lentils) have also shown that the duration of the period of exponential growth declines as sowing date is advanced from autumn to spring (McKenzie, 1987). This is because the duration and rate of growth are usually inversely related (Littleton *et al.*, 1979a). Increasing temperatures in late spring and early summer (Table 4.1) therefore increased the rate of growth in the September sowing, hence reducing the duration of the period of exponential growth (Table 6.3).

6.7.6 Leaf area

The development of leaf area in chickpea is limited early in the growing season when temperatures are low. In this field study, the July sowing only had an LAI of 1 at 144 DAS (Table 6.4). A similar result has been reported at Hisar in northern India where the LAI of chickpea remained less than 1 for the first 100 DAS (Khanna-Chopra and Sinha, 1987). This slow increase in leaf area occurs because the development of leaf area depends on the rate of appearance and expansion of individual leaves both of which increase with increasing temperature (Littleton *et al.*, 1979a). Further evidence obtained from *Vicia faba* (Husain *et al.*, 1988b), soybeans (Turner and Burch, 1983) and a range of temperate cereals (Hay and Walker, 1989) confirm the influence of temperature on leaf area development.

Only sowing date had a significant ($p<0.001$) effect on LAI early in the growing season (Table 6.4). This was due to the longer growth duration of the July sowing. That LAI was not affected by additional N early in the growing season has also been reported in lentils sown in Canterbury (Turay, 1993). It also confirms the suggestion by Thomas *et al.* (1987) that growth at low temperature is unlikely to be limited by nitrogen. As temperatures became more favourable in November-December, the influence of additional N on LAI became apparent. The LAI of plants which had received 90 kg N/ha was 36% higher than that of the unfertilized plants (Table 6.4). A similar increase in LAI with additional N has been obtained in other chickpea experiments in Canterbury (eg. McKenzie *et al.*, 1992). It is attributed to enhanced branching giving rise to more leaves (Summerfield *et al.*, 1977; Rawsthorne *et al.*, 1985a).

The maximum LAI of 3.2 obtained in this field trial is less than the 3.5 reported by Hernandez and Hill (1985) but is within the range of 3 to 4 normally encountered in unirrigated chickpea in the more traditional chickpea growing areas (Saxena *et al.*, 1983). The increase in leaf area index was faster in the September sowing which attained its maximum LAI much earlier (85 DAS) than the July sowing (174 DAS). This telescoping of growth development is commonly observed in temperate cereals and results in lower maximum leaf area indices as seen in the present study (Hay and Walker, 1989). Towards the end of the growing season, leaf senescence was delayed by additional N (Table 6.4). This is an indication that leaf senescence is not determined entirely by the phenological stage of the plants and that other environmental factors including the nitrogen nutrition of the plant play a major role (Littleton *et al.*, 1979a; Sheldrake and Saxena, 1979; Squire, 1990).

6.7.7 Photosynthetically active radiation intercepted (PAR)

Photosynthetically active radiation is the flux in the 0.4-0.7μm wave band of the solar spectrum that initiates the flow of energy required in photosynthesis (Hipps *et al.*, 1983; Howell *et al.*, 1983). It is almost independent of atmospheric conditions and is nearly a constant fraction of total solar radiation (Monteith, 1972; Szeicz, 1974b). In this study, both maximum and total PAR intercepted was significantly influenced by sowing date and N application. Maximum PAR intercepted increased with increasing N application. The plants

provided with 90 kg N/ha intercepted 7% more maximum PAR compared to those receiving no additional N. At the end of the growing season these plants had intercepted 5% more PAR (760 MJ PAR/m^2 against 725 MJ PAR/m^2) (Table 6.5). Similar increases in total PAR intercepted with additional N have been reported in lentils and chickpeas grown in Canterbury (McKenzie *et al.*, 1992; Turay, 1993).

Because the amount of PAR intercepted increased with increasing LAI, the significantly higher maximum and total PAR intercepted by the plants receiving additional N can be attributed to the greater maximum LAI attained and the longer leaf area duration (Table 6.4). This has also been reported by other workers (Monteith, 1977; Gallagher and Biscoe, 1978). Although it significantly affected maximum PAR intercepted, sowing date had a more profound influence on the total PAR intercepted. Photosynthetically active radiation intercepted declined as sowing date was advanced with the July sowing intercepting 20% more PAR (810 MJ PAR/m^2) than the September sowing (675 MJ PAR/m^2) (Table 6.5). This amount of PAR intercepted is close to the 778 MJ PAR/m^2 and 654 MJ PAR/m^2 intercepted by autumn and spring sown lentils respectively in Canterbury (Turay, 1993). It is however higher than the 519 MJ PAR/m^2 and 439 MJ PAR/m^2 intercepted by winter and spring sown chickpeas respectively in northern Syria (Hughes *et al.*, 1987). In that study, the plant population used was lower (30 plants/m^2) than that used in the present field trial and the greater than average rainfall received during spring may have led to less radiation being intercepted. Since the duration of growth has the most influence on the amount of solar radiation intercepted (Husain *et al.*, 1988b; Lawn, 1989), early sowing enabled the crop to intercept more PAR as seen in this study and that by McKenzie and Hill (1990) with lentils.

6.7.7.1 Dry matter accumulation and PAR intercepted

Since PAR is nearly constant at 0.5±0.03 (Szeicz, 1974b), numerous attempts have been made to analyse crop growth in terms of the amount of PAR intercepted during the growing season and its efficiency of conversion into dry matter (Shibles and Weber, 1965; Williams *et al.*, 1965). Available evidence indicates that the rate of conversion of intercepted PAR to dry matter is a conservative quantity and the amount of PAR intercepted is the variable that determines crop growth (Hughes *et al.*, 1987). During their vegetative growth therefore, crops

accumulate dry matter at rates which are proportional to intercepted radiation (Monteith, 1977). Figure 14 shows the close relationship between intercepted PAR and dry matter accumulation at both sowing dates. The regressions accounted for 98 and 99.4% of the variance in the September and July sowings respectively. Early in the growing season, a few data points in the July sowing lie below the fitted line (Figure 14). This is because at that stage LAI was small and crop net photosynthesis is saturated at lower irradiance, hence, dry matter production per unit of absorbed PAR was small (Gallagher and Biscoe, 1978; Husain *et al.*, 1988b). These results support Hernandez and Hill's (1985) finding that differences in dry matter production from chickpeas in Canterbury were related to the amount of solar radiation intercepted during the growing season. Similar results have also been reported in wheat and barley (Gallagher and Biscoe, 1978), cowpeas (Littleton *et al.*, 1979b), pigeon pea (*Cajanus cajan* (L.) Millspaugh) (Hughes and Keatinge, 1983), chickpea (Hughes *et al.*, 1987), field bean (Husain *et al.*, 1988b) and lentils (McKenzie and Hill, 1991).

The average utilization coefficient of 1.64 g DM/MJ PAR intercepted did not vary significantly with any factor and is close to the 1.6 g DM/MJ PAR absorbed obtained in lentils in Canterbury (McKenzie and Hill, 1991). However, it is higher than the utilization coefficient of 0.91 g DM/MJ PAR intercepted in chickpeas reported by McKenzie *et al.* (1992). The weed problem in their study is partly responsible for the lower utilization coefficient. Generally, utilization coefficients in grain legumes are lower than those of cereals because of the higher protein and lipid content in grain legume seeds requiring more photosynthate (Monteith, 1972; Tanaka, 1983) and the energy cost of N fixation (Pate *et al.*, 1988).

6.8 Conclusions

The seed yield results indicate that winter sowing of chickpea in Canterbury is unlikely to provide the benefits of winter sowing of chickpea in the Mediterranean region because harvest index declines with early sowing. If farmers are to derive maximum benefit from chickpea, they should sow their crop in late September or early October. There were no yield increases in the field with the application of nitrogen and hence added fertilizer nitrogen cannot be recommended.

CHAPTER 7

GENERAL DISCUSSION

7.1 Nodulation

Surveys in the Indian sub-continent, Mediterranean region and North Africa; regions where chickpea has been under cultivation for millennia indicate variability in its nodulation (Ibrahim and Salih, 1980; Islam, 1981b; Khating and Ghonsikar, 1981). Most investigators therefore recommend inoculation of chickpea with the appropriate rhizobial strain because it is a cheaper and possibly more effective way of providing adequate nitrogen supply (Evans, 1982; Nambiar, 1990). While competition with native rhizobial strains may account in part for the variability in nodulation in the more traditional chickpea growing areas (Rupela and Beck, 1990), the poor nodulation in the present study can be attributed to the low soil temperatures during germination and early vegetative growth. Barrios *et al.* (1963) and Andrews (1986b) have reported nodulation failure in *Phaseolus vulgaris* and *Vicia faba* at 12 °C and 10 °C respectively. These temperatures are well above those recorded in the early part of the 1992/93 growing season (Table 4.1), and it is conceivable that these low temperature may have inhibited nodulation in one way or the other. Further support is provided by the more favourable nodulation response to inoculation during the 1993 season (Figure 4) when mean daily soil temperatures from August to October were 2 °C higher than they were at a similar period in the previous season (Table 4.2).

Results from the field (1992/93) and glasshouse experiments (Table 5.1 and 5.2) confirm the significant reduction in nodule number in the presence of additional nitrogen already reported in other legumes (Dazzo and Truchet, 1984; Streeter, 1985a,b; Kijne, 1992). Whether nitrate inhibition of legume nodulation is due to its effects on the host plant or the bacterial strain is not known (Gibson and Harper, 1985). However, it is clear that comparison of experiments on the effects of nitrate on nodulation can be hampered by the use of different concentrations and durations of nitrate treatments (Becana and Sprent, 1987). It is generally accepted that excess nitrate may depress nodulation by reducing the number of infection sites or the number of successful infections (Pate and Dart, 1961). Data in Table 5.2 supports this assertion as

N application significantly reduced root dry weight at 30 and 45 DAS.

Under field conditions, the effects of additional N on nodule number diminished as the growing season progressed (Table 5.1) as reported earlier by Hernandez (1986). Whether this recovery in nodule number can confer beneficial effects in terms of fixed nitrogen late in the growing season when the demand for fixed nitrogen is greatest remains to be determined. That the effects of additional N persisted throughout the growth duration in the glasshouse experiment suggests that caution should taken when extrapolating such results into the field. Glasshouse results on the effects of water stress on nodule number (Table 5.2) were in general agreement with those obtained in soybean and field bean (Sprent, 1971, 1972a,b; Gallacher and Sprent, 1978).

7.2 Growth

Experimental evidence on the effects of various environmental factors on seed reserve mobilization in grain legumes is scanty. Data in Table 5.2 indicates that seed reserve mobilization was limited by moisture stress. This occurred because plant growth which depends on cell division and enlargement was restricted by moisture stress hence limiting the demand for metabolites (Squire, 1990). That additional N enhanced seed reserve mobilization (Table 5.2) was expected because it is associated with increased cell division and enlargement (Hay and Walker, 1989); processes that require the supply of metabolites which could only be met by greater mobilization of seed reserve. It can be deduced from these findings that increased seed reserve mobilization was a possible mechanism by which supplemental watering and additional N enhanced early shoot growth. Early root growth in the glasshouse trial seems to have responded rather anomalously to additional N. While other workers (eg. Das, 1982; Jessop *et al.*, 1984) have observed increases in root dry weight with added N, data in Table 5.3 shows the reserve. This result can be attributed in part to a reduction in dry matter investment in the roots when N source was assured. Coupled with the respiratory burden of reducing nitrate in the roots, it becomes clear why additional N reduced root growth.

In the field, leaf area increase during early growth was mainly influenced by sowing date with the July sowing having a greater LAI because of the longer growth duration. Results in Table

6.4 indicates that the influence of additional N on LAI was dependent on favourable temperatures; a conclusion that has already been reached in *Vigna unguiculata* (Littleton *et al.*, 1979a), *Phaseolus vulgaris* (Thomas *et al.*, 1987) and *Lens culinaris* (Turay, 1993). Green area per plant in the glasshouse increased with additional N (Table 5.4) confirming the field trial results. Because the glasshouse experiment was carried out in greater detail, it was possible to attribute the increase in green area per plant to an increase in branch and leaf number per plant (Table 5.3 and 5.4). These factors are thought to have contributed to the increase in LAI in the field as has been reported in other chickpea studies (eg. Rawsthorne *et al.*, 1985a). Leaf senescence and ontogenetic decline in leaf size along the canopy are factors that contribute to leaf area decline (Squire, 1990). This was confirmed by the results from this study. Over a given period of time, leaf number increase in the glasshouse was greater than the corresponding increase in green area (Table 5.4) indicating that individual leaf size had decreased along the canopy.

The glasshouse provided a controlled environment under which the effects of water stress on chickpea growth could be studied more closely as recommended by Hernandez (1986). Supplemental watering increased green area per plant throughout the growth duration (Table 5.4). Like the increases in green area per plant with additional N, supplemental watering increased green area per plant by enhancing branching and number of leaves per plant (Table 5.3 and 5.4). Water stress affected green area per plant more than leaf number per plant, a finding already reported in six other grain legumes (Muchow, 1985). However it was clear that the full potential of additional N to increase leaf area per plant could not be realised if water was limiting (Figure 10) because water stress affects nutrient uptake and translocation (Slayter, 1973).

As there is seldom a complete lack of mineral nitrogen in the field, differences between plants dependent on nitrogen fixation and those dependent on nitrate are difficult to detect (Sprent *et al.*, 1988). This was observed in leaf area which did not respond to rhizobium inoculation in the field (Table 6.4) but under glasshouse conditions was significantly increased at 45 and 60 DAS (Table 5.4). Attempts to make these differences more apparent by depleting plant available nitrogen have largely been unsuccessful (Corbin *et al.*, 1977). Saxena *et al.* (1983) and Singh (1991) have reported dry matter increases in chickpea following irrigation. While supplemental watering increased shoot dry weight/plant in the glasshouse experiment (Table

5.5), it is unlikely that such increases will be reflected in increased seed yield under Canterbury conditions because the combination of stored soil moisture and rainfall received may be sufficient for crop growth (McKenzie and Hill, 1990). Shoot percent N of the glasshouse grown chickpeas was significantly affected by N application and *Rhizobium* inoculation. Inoculation with the recommended rate of inoculum contributed to the shoot an amount of N equivalent to that obtained by assimilating 90 kg N/ha of nitrate (Figure 12).

Dry matter accumulation in the field increased as LAI increased (Tables 6.1 and 6.4) confirming the dependence of dry matter accumulation on leaf area increase (Saxena N.P., 1984). Both LAI and DM accumulation were strongly influenced by temperature as already reported in *Vicia faba* (Husain *et al.*, 1988b) and lentils (Turay, 1993) grown in Canterbury. Sowing date therefore had a profound effect on maximum dry matter accumulated (Table 6.1). Nitrogen application significantly increased LAI in the field but the increase was not reflected in increased total dry matter accumulation (Table 6.1 and 6.4). A similar result has been obtained in chickpea experiments in India (Saxena and Sheldrake, 1980). There were no differences in the total dry matter accumulated by chickpea dependent on nitrogen fixation or nitrate. This may provide support to the claim that data indicating better performance of nitrate dependent plants was likely to have been obtained under growing conditions optimised for nitrate (Sprent and Thomas, 1984).

Total PAR intercepted in the field over the growing season was similar to that obtained in other experiments in Canterbury (Husain *et al.*, 1988b; Turay, 1993). Additional N and sowing date significantly affected maximum and total PAR (Table 6.5). This occurred because additional N increased LAI and leaf area duration (Table 6.5), critical factors that determine the amount of solar radiation that a canopy can intercept (Monteith, 1977). The July sowing was also in the field for a longer duration and therefore intercepted more solar radiation (McKenzie and Hill, 1990).

Analysing crop growth in terms of the amount of solar radiation absorbed and its efficiency of conversion into dry matter is physiologically more relevant than the traditional methods (Gallagher and Biscoe, 1978). Estimates of dry matter accumulated by a range of crops agree well with measurements of PAR intercepted during the vegetative growth stage (Monteith, 1972; Littleton *et al.*, 1979b). The results obtained in this study are consistent with those

from other chickpea experiments in Canterbury which indicate a strong linear relationship between intercepted PAR and total dry matter accumulation throughout the growing season (Hernandez, 1986; McKenzie *et al.*, 1992). Utilization coefficient did not vary with any factor, confirming other reports that it is a conservative quantity (Hughes *et al.*, 1987).

7.3 Seed yield, yield components and harvest index

While N application and sowing date had significant effects on LAI and hence PAR intercepted, seed yield at final harvest did not respond to any factor (Table 6.1). Siddique and Sedgley (1986) have obtained similar results and concluded that early sown chickpeas over invested most of their dry matter into stem and leaf production. With its poor partitioning of dry matter into economic yield, seed yield therefore failed to respond to both factors (Khanna-Chopra and Sinha, 1987). The seed yield obtained is comparable to that from other chickpea experiments in the region but is lower than that obtained in the Mediterranean region especially when sowing is done in winter (Saxena M.C., 1980, 1984). Because of the low harvest index in Canterbury with early sowings (McKenzie and Hill, 1994), seed yield increases with winter sowing are unlikely.

Variations in seed yield are almost always due to differences in the number of pods per unit area (Saxena and Sheldrake, 1980; Saxena *et al.*, 1983). This was not the case in this study because the July sowing had 29% more pods per plant than the September sowing yet seed yield in both sowing dates was similar. In the July sowing, the significantly ($p<0.05$) lower seed per pod may have jeopardised any benefits in seed yield that could have accrued from the significantly ($p<0.001$) higher number of pods per plant (Table 6.2). The thousand seed weight was not affected significantly by any treatment confirming its stability (Littleton *et al.*, 1979b).

Conclusions

1. *Rhizobium* inoculation did improve nodulation in the field. However, under all circumstances nodulation was less than expected and there were no yield responses to inoculation.

2. Farmers are unlikely to obtain a yield advantage with winter sowing but sowing in late September or early October will give maximum yields.

3. Additional N reduced the number of nodules initiated by chickpeas grown under field and glasshouse conditions, albeit only during the early part of the growing season in the field.

4. Added fertilizer N did not significantly increase either DM or seed yield in this study.

5. On the basis of percent N accumulated in the shoot, nitrogen fixation in chickpea can provide an amount equivalent to that derived by nitrate dependent plants provided with 90 kg N/ha.

6. In the glasshouse, water stress reduced nodulation and dry matter production primarily through reducing green area.

Further research

1. The effectiveness of *Rhizobium* strain CC1192 commonly used to inoculate chickpea in Australia and New Zealand should be studied more closely. Comparisons with other rhizobial strains should be made and commonly used methods of rhizobial inoculation also examined.

2. Attempts should be made to quantitatively estimate the amount of nitrogen fixed by chickpeas under the Canterbury environment.

3. Further studies of nitrogen responses on low fertility soils would help quantify the responses of chickpea to added nitrogen.

4. Intensive irrigation studies are needed to accurately assess the effects of water stress in the field on growth and yield of chickpeas.

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APPENDIX

Appendix 1: Standard errors of the means for the number of nodules per plant in chickpeas sown on 3 dates in Canterbury, 1993.

	Sample number				
Sowing date	1	2	3	4	5
July	1.23	1.77	2.14	2.39	2.00
August	1.77	2.14	2.39	2.00	-
September	2.14	2.39	2.00	-	-